Review Article

Oral Manifestations of Systemic Diseases in Cats

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Abstract

Oral lesions in cats are common disorders that adversely affect quality of life. Certain conditions (eg, stomatitis) may develop very early in life in some purebred cats. These lesions are frequently encountered in geriatric cats and in individuals that are immunosuppressed or have chronic systemic disease. Immunosuppression facilitates the involvement of opportunistic agents, disrupts the microbiota, and contributes to the development of oral lesions. In addition, infectious diseases, metabolic disorders, and multifactorial etiological factors are implicated in their pathogenesis. Many systemic diseases have oral manifestations. These manifestations can be categorized, based on histopathological/clinical features and etiology, into stomatitis, gingivitis, gingivostomatitis, chronic ulcerative stomatitis, lymphoplasmacytic gingivitis/ stomatitis, and feline chronic gingivostomatitis.

Keywords: Oral lesions, feline, feline chronic gingivostomatitis (FCGS), immunosuppression.

Introduction

Oral lesions are serious disorders that significantly impair quality of life and may ultimately lead to death. These lesions often present with common clinical signs. Halitosis is typically associated with volatile sulfur compounds produced by anaerobic bacteria secondary to periodontal disease and plaque accumulation; however, it may also occur in association with gingivitis, periodontitis, stomatitis, bacterial infections, oral neoplasms, chronic viral diseases, oronasal fistula, upper respiratory tract disease, chronic rhinitis, uremic oral lesions secondary to renal failure, eosinophilic granuloma complex, diabetes mellitus, and gastrointestinal disorders. Chronic drooling and ptyalism are most commonly related to oral irritation or dysphagia rather than increased salivary production, and are associated with oral infections, neoplasia, oropharyngeal inflammation, foreign bodies, upper respiratory tract diseases, and toxicity (Clarke and Caiafa, 2014).Additional clinical signs may include dysphagia (dropping food from the mouth, refusal of dry food), anorexia, retching, sneezing, nasal discharge, protrusion of the tongue, pain on opening the mouth, reduced grooming, oral bleeding, and facial asymmetry (Falcão et al., 2020; Wolf, 2006). Oral examination is an integral component of every health assessment and includes evaluation of presenting complaints, general and

extraoral examination, intraoral examination without anesthesia, and, when necessary, detailed assessment under anesthesia. In cats, oral lesions may be inflammatory or neoplastic, and histopathological evaluation is required for definitive diagnosis (Falcão et al., 2020).In metabolic or endocrine disorders, immunosuppression contributes to lesion development. Infections such as Feline Leukemia Virus (FeLV), Feline Immunodeficiency Virus (FIV), Feline Calicivirus (FCV), Feline Infectious Peritonitis (FIP), Feline Herpesvirus Type 1 (FHV-1), panleukopenia virus, Feline Coronavirus (FCoV) and *Hemobartonella henselae*, as well as nutritional disorders, are also associated with oral lesions, and breed predispositions have been reported (Lyon, 2005).

Classification of Oral Lesions

In cats, oral lesions can be classified into the following subcategories: stomatitis, gingivitis, gingivostomatitis, chronic ulcerative stomatitis, lymphoplasmacytic gingivitis/stomatitis, and feline chronic gingivostomatitis.

Stomatitis

Stomatitis is an inflammation of the oral mucosa that may also involve the gingiva and tongue. It is distinguished from gingivitis and periodontitis by its extension to non-gingival mucosal tissues and is typically characterized by marked mucositis in the caudal oral cavity. The reported prevalence in cats ranges from 0.7% to 12% (Jennings et al., 2015).The etiology includes dental calculus, periodontal disease, and infectious agents. Feline rhinotracheitis and FCV are associated with acute stomatitis, whereas FeLV and FIV are linked to chronic forms. In immunosuppressed cats, overgrowth of Candida spp., as well as uremia in certain metabolic disorders, may also contribute to the development of stomatitis. Clinical findings include anorexia, hypersalivation, hyperemia of the oral mucosa, epithelial friability, halitosis, and pain.

Gingivitis

Gingivitis is an inflammation of the gingiva surrounding the teeth. In affected patients, the gingiva is hyperemic and prone to bleeding; in chronic stages, gingival recession may occur, leading to root exposure. The inflammatory process may extend to the gingiva, lips, periodontal region, and other oral tissues. (Figure 1) It commonly develops secondary to alveolar periostitis and dental calculus and may result in clinical signs such as anorexia, salivation, reluctance to eat, and halitosis. The primary cause is plaque-forming bacteria that colonize the gingival sulcus. These bacteria and their by-products penetrate from the sulcular epithelium into the underlying connective tissue, eliciting an inflammatory response. Gingivitis may be acute or chronic. Chronic stimulation can lead to gingival hypertrophy or proliferation. The condition may resolve if the underlying cause is eliminated; however, if left untreated, it can progress to periodontitis (Southerden, 2010). Gingivitis typically precedes and accompanies periodontitis. As the disease progresses, the integrity of the gingiva is compromised, ulceration develops within the sulcus, and bacteria are allowed to invade deeper periodontal structures. In cases of immunosuppression, the progression from gingivitis to periodontitis may be accelerated (Lyon, 2005).



Figure 1: Severe gingival banding in a cat (Original)

Gingivostomatitis

Gingivostomatitis is a common pathological condition, typically characterized by submucosal infiltration of plasma cells. Increased numbers of neutrophils, lymphocytes, and macrophages are also observed. Immunoglobulin levels are elevated in both serum and saliva. The immune response is evaluated based on T-lymphocyte subsets, and the CD4/CD8 ratio is generally decreased due to elevated CD8 levels, indicating an aggressive immune response.In cats, increased expression of cytokine genes, particularly elevated interleukin-4 (IL-4) levels, has been reported. A potential viral association with FCV, FHV, FIV, and FeLV has been suggested, and gene transfer has also been considered as a possible contributing factor. Although the exact etiology remains unclear, gingivostomatitis is defined as a multifactorial

disease involving bacterial, viral, genetic, nutritional, environmental, and domestication-related factors (Lyon, 2005).



Figure 2: Gingivostomatitis in a cat (Original)

Chronic Ulcerative Stomatitis

Chronic Ulcerative Stomatitis (CUS) is a mucocutaneous disorder primarily affecting mucosal surfaces and is characterized by erosive and ulcerative lesions. The tongue is the most commonly affected site, followed by the buccal mucosa and gingival tissues. Less frequently, lesions may be observed on the labial mucosa or hard palate. Clinical signs include halitosis, anorexia, pain-associated vocalization during eating or yawning, approaching the food bowl without eating, and salivation. Varying degrees of inflammation, proliferation, and ulceration are observed in the affected gingiva and oral mucosa. The mucosa is typically bright red, the tissues are fragile, and bleeding occurs easily. The inflammatory process is most often bilateral (Niemiec and FAVD, 2014).

Lymphoplasmacytic gingivitis/stomatitis

Feline lymphocytic–plasmacytic stomatitis (LPGS), also referred to as caudal stomatitis, is an inflammatory disease characterized by painful erosive lesions and proliferative changes in the oral mucosa. This persistent oral condition, with an incidence exceeding 3%, leads to severe inflammation and painful erosive lesions, particularly in the buccal region and caudal oral mucosa. Cats may develop the disease at an early age and may require lifelong management. Clinical signs include ptyalism, pain, halitosis, bleeding, weight loss, and dysphagia, and the condition may necessitate euthanasia due to markedly reduced quality of life. Genetic predisposition, environmental stress, nutritional factors, abnormal immunological responses, and viral and bacterial infections have all been associated with LPGS (Hung et al., 2014). Although LPGS is most commonly observed in middle-aged and older cats, it may occur as early as 6 months of age in Abyssinian, Somali, and certain purebred cats. Lesions may develop along the gingival margins and may be associated with Feline Odontoclastic Resorptive Lesions (FORL). In the presence of FORL, proliferative gingival tissue may be observed. In more severe cases, marked proliferative and painful hyperplastic tissues may be present within the oral cavity. Occasionally, polypoid lesions may develop on the inner surfaces of the lips. Lesions are typically bilateral, a feature that is important in differentiating this condition from oral neoplasms. In the absence of concurrent disease, complete blood count findings are usually unremarkable; however, biochemical profiles often reveal hyperglobulinemia and hyperproteinemia, which are attributed to immunological responses and lymphoid proliferation (Wolf, 2006). Histologically, the disease is characterized by a diffuse oral inflammatory response with ulcerative–proliferative lesions associated with lymphoplasmacytic cell infiltration. Although the exact etiology remains unclear, the development of oral lesions is thought to be associated with inflammation driven by an exaggerated immune response (Fontes et al., 2023).

Feline Chronic Gingivostomatitis

Feline chronic gingivostomatitis (FCGS) is a destructive, chronic, and complex inflammatory mucosal disease affecting approximately 26% of domestic cats. It causes severe oral pain and may be potentially life-threatening in at least 10% of cases (Soltero-Rivera et al., 2024). Affected cats may exhibit decreased appetite, dysphagia, and weight loss (Southerden, 2010). The disease may manifest in three stages: early gingival inflammation during the eruption of deciduous teeth, inflammation triggered by the eruption of permanent teeth, and the most common form observed in cats with a mean age of 7–10 years (Abbasi et al., 2025). It is more frequently reported in Persian, Siamese, Abyssinian, Himalayan, and Burmese breeds, with no reported sex predilection (Sánchez-Vallejo et al., 2025).Recurrence is common, and effective treatment options are limited. Surgical intervention, including extraction of premolar and molar teeth, is often recommended to reduce bacterial load and control inflammation. Ulcerative and/or proliferative lesions are typically localized lateral to the palatoglossal folds (Lee et

al., 2020). Lesions are usually bilateral and are most commonly observed in the glossopalatine arches, fauces, and alveolar mucosa adjacent to the teeth.(Figure 3) Less frequently, they may be found at the base of the tongue, pharynx, palate, and rostral regions of the lips (Fontes et al., 2023). Clinically, proliferative and ulcerative phenotypes are observed. In advanced cases, ulceration, erosion, and marked inflammation of the tongue may develop; tongue lesions can impair eating and drinking, and in some cases, proliferative tissues may prevent tongue retraction (Lee et al., 2020; Sánchez-Vallejo et al., 2025).



Figure 3: Images of Feline Chronic Gingivostomatitis in 2 Different Cats (Original)

The pathogenesis may involve bacteria (*Pasteurella multocida, Bartonella spp., Fusobacterium nucleatum*), viruses (FIV, FHV1, FCV, FeLV), periodontal disease, and upper respiratory tract infections. In addition, $CD8^+$ T cell–mediated immune responses, IL-6 and IL-17–associated pathways, and myeloid cells contribute to disease development; alterations in the oral microbiome facilitate pathogen colonization and modulate the local immune response (Sánchez-Vallejo et al., 2025). Histopathologically, FCGS is characterized by lymphoplasmacytic infiltration. Immunohistochemical studies demonstrate $CD3^+$ T lymphocyte infiltration in the epithelium and subepithelial stroma, whereas $CD20^+$ B lymphocytes are largely confined to the subepithelial stroma. Increased $CD8^+$ T cell levels and a decreased CD4/CD8 ratio suggest a cytotoxic cell-mediated response and immune dysregulation (Lee et al., 2020).In the chronic phase, antibodies derived from plasma cells may activate the complement system, leading to damage of the stratified squamous epithelium, increased vascular permeability, and gingival recession. The typical distribution of oral lesions often supports clinical diagnosis. The disease is a multifactorial inflammatory condition that develops as a result of an inappropriate host immune response to chronic oral antigenic stimulation (Druet and Hennet, 2017).

Infectious Causes of Oral Lesions

Feline Calicivirus Infection (FCV)

Feline calicivirus (FCV) is a virus belonging to the genus Vesivirus within the family Caliciviridae (Hurley and Sykes, 2003). It is widespread and highly contagious within the feline population. FCV can cause severe pneumonia in kittens and, less commonly, in adult cats. It has been associated with feline chronic gingivostomatitis, and infection with virulent systemic FCV (VS-FCV) may result in epizootic spread, particularly in multi-cat environments, and is often fatal (Hofmann-Lehmann et al., 2022).Clinical manifestations vary depending on the viral strain, age, and husbandry conditions. The most common findings include oral ulcerations, particularly on the tongue and palate, along with mild upper respiratory tract signs (Hurley and Sykes, 2003).(Figure 4)



Figure 4: Linguinal ulcer in two separate cats (Original)

While infection may remain subclinical in some cats, others may develop the characteristic lingual ulcer syndrome (Hofmann-Lehmann et al., 2022). An association has been demonstrated between FCV and the lymphoplasmacytic gingivostomatitis (LPGS) complex, with FCV positivity reported in approximately 80% of affected cats (Radford et al., 2007).

Feline Herpesvirus Infection (FHV-1)

The causative agent of viral rhinotracheitis in cats is widespread worldwide and is currently designated as Felid alphaherpesvirus-1 (FHV-1) (Gaskell et al., 2007). The natural routes of entry for FHV-1 are the nasal, oral, and conjunctival mucosa.

Following infection, viral replication during the acute phase occurs primarily in the mucosa of the nasal septum, turbinates, nasopharynx, and tonsils. The conjunctiva, mandibular lymph nodes, and upper trachea are also frequently involved. Infectious virus can be detected in oropharyngeal and nasal swabs approximately 24 hours after infection (Gaskell et al., 2007).FHV-1 is shed in ocular, nasal, and oral secretions, and transmission occurs predominantly through direct contact. Both acutely infected cats and carrier cats represent important sources of infection. Early clinical signs include depression, sneezing, anorexia, and hyperthermia, followed by ocular and serous nasal discharge. Although oral ulceration may occur in FHV-1 infection, it is less common compared with FCV infection (Gaskell et al., 2007).

Feline Immunodeficiency Virus (FIV)

Feline immunodeficiency virus (FIV) is an enveloped RNA virus belonging to the genus Lentivirus within the family Retroviridae (Sykes, 2013). Adult cats are more susceptible than kittens. The virus is present in saliva and is most commonly transmitted through bite wounds. In addition, transmission may occur via blood, saliva-contaminated fomites, grooming of infected animals, as well as through the placenta and milk. Free-roaming male cats and older individuals are at increased risk.The virus replicates in lymphoid tissues and salivary glands and subsequently disseminates throughout the body. As long as the immune system remains competent, clinical signs may not be evident for years. Clinical manifestations are typically observed in the terminal stages and are often associated with secondary disorders, and infected cats remain lifelong carriers. In immunosuppressed cats, infections such as FCV, FHV-1, *Toxoplasma spp., Chlamydia spp.,* and *Hemobartonella spp.* may occur. In these animals, stomatitis, gingivitis, and gingival banding are commonly observed (Figure 5). Some FIV strains exhibit high replication in lymphocytes with limited replication in macrophages, whereas others replicate similarly in both cell types; these differences are associated with variations in clinical presentation (Hartmann, 1998).



Figure 5: Gingival banding in an FIV-positive cat (Original)

Feline Leukaemia Virus (FeLV)

Feline leukemia virus (FeLV) is an enveloped RNA virus belonging to the genus Gammaretrovirus within the family Retroviridae. Three major subtypes have been identified: FeLV-A, FeLV-B, and FeLV-C, each utilizing different cellular receptors for entry. All cats infected with FeLV-B or FeLV-C are also infected with FeLV-A, and FeLV-A is the only subtype transmissible between cats. These subtypes may influence the clinical course of the disease.Clinically, FeLV infection is characterized by immunodeficiency, including leukopenia, lymphopenia, varying degrees of anemia, progressive weight loss, persistent diarrhea, and opportunistic or secondary infections. Chronic and recurrent bacterial or viral upper respiratory tract infections, gingivitis, chronic cutaneous infections, FCV, and FIP may also be observed. In progressive FeLV infection, clinical findings vary depending on the stage of disease and the presence of secondary conditions. While some cats remain asymptomatic, others may exhibit hypothermia, mucosal pallor, petechial hemorrhages, dehydration, peripheral lymphadenomegaly, cachexia, stomatitis, gingivostomatitis, subcutaneous abscesses, and upper respiratory tract signs.The principal clinicopathological abnormalities associated with retroviral infections include anemia, leukopenia, neutropenia, lymphopenia, decreased albumin-to-globulin ratio, and hypergammaglobulinemia. The clinical and pathological features of FeLV and FIV syndromes largely overlap and cannot be reliably distinguished based solely on clinical findings. In cases of co-infection, a synergistic effect on immunosuppression and disease progression may be observed (Hoover and Mullins, 1991). In FeLV infection, opportunistic infections may develop as a result of myelosuppression or acquired cell-mediated immunodeficiency. Impaired neutrophil function further exacerbates the effects of neutropenia, predisposing affected cats to infections such as bacterial infections of the upper and lower urinary tract, hemoplasmosis,

upper respiratory tract infections, FIP, chronic stomatitis, toxoplasmosis, dermatophytosis, and cryptococcosis (Sykes and Hartmann, 2013).

Candidiasis

Oral candidiasis is a fungal infection most commonly caused by *Candida albicans*. The organism is a normal opportunistic component of the flora of the oral mucosa, nasopharynx, gastrointestinal tract, and external genital tract. Although rare in cats, it may occur in regions such as the lips and tongue. It is typically localized to mucocutaneous tissues, remains confined to the epithelium, and does not usually invade deeper structures.The most common predisposing factors include immunosuppression and prolonged antibiotic or corticosteroid therapy. Consequently, it may be more frequently observed in cats undergoing treatment for gingivostomatitis. Oral and perioral candidiasis represent the most common forms of acute mucocutaneous candidiasis. The condition is characterized by small lesions or whitish papules forming a creamy, adherent layer on the tongue, buccal mucosa, and palate. Lesions may occasionally extend to the tongue, palate, and pharynx and reach the commissures of the mouth. Typical findings include white plaques and a tendency for bleeding in the surrounding ulcerated areas (Soto, 2020).

Fungal Cryptococcosis

Cryptococcosis is a deep mycosis that occurs sporadically worldwide in humans and animals and represents the most common systemic fungal infection in cats. The causative agents are most commonly *Cryptococcus neoformans* and *Cryptococcus gattii* (Trivedi et al., 2011). Although the exact mode of transmission is not fully elucidated, infection is believed to occur primarily via inhalation. In cats, the nasal cavity is the initial site of infection, and asymptomatic colonization has been reported (Trivedi et al., 2011).The nasal form is the most common presentation and may disseminate to the skin, subcutaneous tissues, bones, and regional lymph nodes. The resulting fleshy mass may protrude from one or both nostrils. Proliferative or ulcerative lesions, as well as gingival proliferation, may be observed in the oral cavity or pharynx. In some cases, oral lesions may be severe enough to interfere with mouth closure and feeding, necessitating surgical removal (Pennisi et al., 2013).

Bartonella Infection

The most commonly isolated Bartonella species in cats is *Bartonella henselae*. Less frequently, infections with *Bartonella clarridgeiae* or *Bartonella koehlerae* may occur. Bartonella species are Gram-negative bacteria that inhabit erythrocytes and endothelial cells. Isolation is typically achieved by bacteriological culture of blood following partial lysis of erythrocytes (Sykes et al., 2010). Domestic cats serve as the primary reservoir for *B. henselae*. Seroepidemiological studies have demonstrated a worldwide distribution of infection, with antibodies detected in 4–80% of cats. Cats may remain bacteremic for several weeks to several years, with a higher prevalence observed in younger cats (<1 year of age). Direct transmission in flea-free environments and vertical transmission from queen to kitten have not been demonstrated. Warm and humid climates are associated with increased infection rates and ectoparasite infestations. The cat flea (*Ctenocephalides felis*) plays a critical role in transmission (Chomel et al., 2006).Bartonella infection may be associated with gingivostomatitis and is considered to represent a secondary manifestation related to immunosuppression.

Fusobacterium Infection

Fusobacteria are Gram-negative, non-spore-forming, rod-shaped anaerobic bacteria that produce butyric acid as the primary end product of fermentation. *Fusobacterium necrophorum* is an opportunistic pathogen that is part of the normal flora of the oral cavity and gastrointestinal tract and is one of the most commonly isolated anaerobes from abscesses and respiratory tract infections in animals. *F. necrophorum* is associated with a range of necrotic conditions collectively referred to as necrobacillosis. This condition most commonly affects the liver, feet, mammary glands, as well as the oropharynx and oral mucosa. In the oral cavity, it may result in caudal stomatitis characterized by focal lesions ranging in color from white to brown (Nagaraja et al., 2005).

Metabolic Factors

Uraemia and Azotaemia

Uremia is a clinical syndrome characterized by increased blood urea levels and is associated with renal failure and lower urinary tract diseases. It develops as a result of decreased excretion of metabolites in the urine and is accompanied by systemic lesions that disrupt fluid balance and enzymatic processes. The severity and distribution of uremic lesions may vary among species and are generally related to the duration of exposure to uremic toxins (Ambrosio

et al., 2020).In uremia resulting from tubular necrosis, accumulated toxins lead to parenchymal damage and ischemia. Degeneration of the tubular epithelium occurs, and desquamated cells obstruct the tubular lumen. Increased intraluminal pressure triggers tubuloglomerular feedback, leading to afferent arteriolar constriction and a reduction in glomerular filtration rate. Consequently, oliguria or anuria develops, resulting in acute renal failure. In advanced stages, the glomerular filtration rate may decrease to less than 5% of normal, leading to signs of intoxication. Accumulation of uremic toxins, along with disturbances in acid–base and water–electrolyte balance, plays a critical role in the syndrome. Urea accumulating in tissues and mucosa is converted to ammonia by bacterial urease activity, causing mucosal irritation and inflammation, and frequently resulting in erosive–ulcerative stomatitis in cats.Azotemia is characterized by increased concentrations of urea, creatinine, and other nitrogenous compounds in the blood and is most commonly associated with impaired renal filtration. It is classified as prerenal, renal, or postrenal. Prerenal azotemia results from decreased renal perfusion; renal azotemia is associated with parenchymal damage and reduced glomerular filtration rate; and postrenal azotemia occurs due to obstruction of urine flow distal to the kidneys. Increased resistance leads to hydronephrosis and elevated tubular pressure, enhancing urea reabsorption; prolonged obstruction damages the tubular epithelium and decreases the urea-to-creatinine ratio. As in uremia, erosive–ulcerative stomatitis may develop secondary to mucosal irritation in azotemia, with lesions typically localized to the caudal oral region.

Diabetes mellitus

Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia, regardless of the underlying cause, and is one of the most common endocrine diseases in cats. Its prevalence varies among populations (Feldman et al., 2014). The disease occurs as type 1 and type 2 diabetes, with immune-mediated type 1 being rare (Nelson and Couto, 2019). The most common form in cats is type 2 diabetes, which develops as a result of a combination of insulin resistance and β-cell dysfunction. In obese cats, the risk of diabetes is increased by approximately 3.9-fold. Clinical signs include polyuria, polydipsia, polyphagia, and weight loss.Oral lesions develop as a consequence of glucotoxicity associated with chronic hyperglycemia. Increased mucosal glucose levels promote bacterial and fungal proliferation, while hyperglycemia impairs neutrophil chemotaxis and phagocytosis, weakening host defenses against anaerobes, *Candida spp.*, and other pathogens. This may result in gingivitis, stomatitis, and glossitis. Microvascular alterations, increased capillary permeability, and impaired perfusion reduce mucosal blood supply, leading to non-healing ulcerative and necrotic lesions.Elevated glucose levels further enhance fungal growth, particularly *Candida albicans*, disrupting the microbial balance; halitosis, plaque accumulation, and tooth loss may occur. Dehydration and reduced salivary defense factors, such as lysozyme, render the mucosa more susceptible to trauma. Increased protein catabolism slows oral tissue regeneration and delays healing. Consequently, gingivitis, ulcerative stomatitis, glossitis, gingival enlargement, periodontal disease, and halitosis may develop.

Multifactorial aetiological factors

In addition to infectious and metabolic factors, multifactorial determinants also play a role in the development of oral lesions (Nunn, 2003). As in humans, the onset and progression of oral lesions in cats are influenced by multiple determinants, including individual characteristics, social and behavioral factors, genetic and systemic influences, features specific to the oral mucosa and microbiota, microbial alterations within dental plaque, and other emerging risk factors.

Age

In humans, aging is associated with stomatitis and periodontal diseases, and this relationship is thought to be linked to progressive periodontal destruction and increased susceptibility to disease over time (Nunn, 2003). Similarly, in cats, advancing age contributes to the development of oral lesions through increased dental plaque accumulation and inadequate oral hygiene.Lymphoplasmacytic gingivostomatitis (LPGS) is most commonly observed in middle-aged and older cats. Feline chronic gingivostomatitis (FCGS) may manifest in three life stages: early gingival inflammation associated with the eruption of deciduous teeth, inflammation triggered by the eruption of permanent teeth, and the most common form observed in cats with a mean age of 7–10 years.

Race and Genetics

Breed represents a complex risk factor for

periodontal disease. In humans, individuals aged 65 years and older of Black ethnicity have been reported to have a higher likelihood of periodontal destruction compared with White individuals, with Prevotella intermedia identified as a risk factor (Nunn, 2003). Differences in the distribution of genetic risk factors among populations may contribute to variations in disease prevalence and severity.Similarly, in cats, certain breeds appear to be predisposed to oral diseases. Lymphoplasmacytic gingivostomatitis (LPGS) is more frequently observed in Abyssinian, Somali, and some purebred cats, whereas feline chronic gingivostomatitis (FCGS) has been reported to be more prevalent in Persian, Siamese, Abyssinian, Himalayan, and Burmese breeds.

Gender

The relationship between sex and periodontal disease is complex. Studies adjusted for oral hygiene, socioeconomic status, and age have demonstrated greater bone loss in males compared with females. In postmenopausal women, estrogen has been suggested to play a role in periodontal disease, with reduced gingival bleeding and inflammation reported in those receiving estrogen therapy (Nunn, 2003). In cats, however, a clear association between sex and periodontal diseases or other oral lesions has not yet been established.

Nutrition

The relationship between nutrition and periodontal disease has been investigated, particularly with respect to calcium and vitamin C intake. According to NHANES III data, low calcium intake is associated with an increased risk of periodontal disease in young men, young women, and older men. Additionally, a study conducted in individuals aged 65 years and older demonstrated that calcium and vitamin D supplementation administered to prevent osteoporosis significantly reduced tooth loss. Within the NHANES III framework, a weak but statistically significant dose–response relationship was identified between vitamin C intake and periodontal disease, with low vitamin C intake reported to increase risk, particularly in smokers (Nunn, 2003).Similarly, in cats, nutritional imbalances may contribute to the development of oral lesions. Wet diets and soft foods may promote tartar formation, thereby accelerating plaque accumulation and the development of infection. Furthermore, vitamin D has been suggested to be associated with gingivostomatitis in cats, and deficiency may represent a risk factor in disease onset; however, further research is required to clarify this relationship (Abbasi et al., 2025).

Social Factors

In humans, socioeconomic status can influence the importance attributed to oral hygiene, and it is well established that smoking and alcohol consumption contribute to its deterioration (Nunn, 2003). Similarly, oral lesions are more frequently observed in cats living in multi-cat households or communal feeding environments. This may be related to the facilitation of transmission of infectious agents through close contact between animals, as well as the generally lower quality of nutrition in such settings.

Microbial Risk Factors

Microbial dental plaque has long been regarded as the primary initiator of periodontal disease. However, not all bacteria within the plaque have been consistently associated with disease progression. The etiological bacteria most commonly identified in periodontal destruction associated with chronic periodontitis include Actinobacillus actinomycetemcomitans, Bacteroides forsythus, and Porphyromonas gingivalis (Nunn, 2003). In a study conducted in cats, the oral microbiota was evaluated, and it was determined that certain microorganisms exhibited increases or decreases in different regions of the oral cavity in cats with feline chronic gingivostomatitis (FCGS).

In conclusion, the recurrent and persistent nature of oral lesions in cats is attributable to the presence of multifactorial etiological factors, including host, agent, and immune-related components. The onset and progression of oral lesions are influenced by numerous determinants, such as individual characteristics, social and behavioral factors, genetic and systemic influences, features specific to the oral mucosa and microbiota, microbial alterations within dental plaque, and other emerging risk factors.Unless the underlying cause is identified and eliminated, lesions tend to recur and become chronic. Therefore, it is essential to address the root cause and establish a treatment protocol accordingly.

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Review Article

Feline Retroviral Infections and Tumour Development: The Oncogenic Roles of Feline Leukaemia Virus and Feline Immunodeficiency Virus

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Abstract

Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are the two most clinically significant retroviruses of domestic cats, both carrying well-documented oncogenic potential. FeLV, a gammaretrovirus, is the most important feline tumour virus known, increasing the risk of lymphoma development up to 60-fold in progressively infected cats through mechanisms including insertional mutagenesis, proto-oncogene activation, and recombination with host cellular oncogenes. FIV, a lentivirus analogous to human immunodeficiency virus (HIV), increases lymphoma risk approximately 5-6-fold, primarily through chronic immune dysregulation, impaired tumour immunosurveillance, and sustained antigenic stimulation of lymphocytes, although direct insertional oncogenesis has also been demonstrated in rare cases. Co-infection with both viruses synergistically amplifies the risk of lymphoid malignancies up to 77-fold. Despite the marked decline in FeLV prevalence following widespread vaccination programmes, the overall incidence of feline lymphoma has not decreased proportionally, suggesting that FeLV-independent aetiological factors including FIV, environmental carcinogens, and chronic inflammation play increasingly important roles. This review provides a comprehensive overview of the oncogenic mechanisms, epidemiological associations, tumour types, and clinical implications of FeLV and FIV infections in domestic cats, with emphasis on recent advances in the understanding of retroviral oncogenesis.

Keywords: Feline leukaemia virus, Feline immunodeficiency virus, Lymphoma, Oncogenesis, Cats

Introduction

Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are retroviruses with a global impact on the health of domestic cats (Hartmann, 2012). Both viruses have been associated with the development of neoplasia, particularly lymphoproliferative malignancies, yet they employ fundamentally different oncogenic strategies. FeLV, a gammaretrovirus, is a directly oncogenic virus capable of transforming host cells through insertional mutagenesis, proto-oncogene transduction, and formation of acutely transforming recombinant viruses (Hartmann, 2012; Neil and Onions, 1985). FIV, a lentivirus structurally and functionally analogous to human immunodeficiency virus type 1 (HIV-1), promotes tumourigenesis predominantly through indirect mechanisms involving chronic immune dysfunction and impaired tumour immunosurveillance (Callanan et al., 1996; Beatty et al., 1998).

The landmark retrospective study by Shelton et al. (1990), encompassing sera from 353 FeLV-infected cats collected over two decades (1968–1988), established a strong correlation between FeLV viraemia and lymphoid malignancies and independently demonstrated a significant association between FIV infection and lymphoproliferative disease. Subsequently, Louwerens et al. (2005) reported that the risk of lymphoma was 62-fold higher in FeLV-positive cats, 5.6-fold higher in FIV-positive cats, and 77-fold higher in cats co-infected with both viruses compared to uninfected controls. These findings underscored the critical role of feline retroviruses in the aetiology of feline lymphoma and provided the epidemiological foundation upon which subsequent mechanistic studies were built.

Despite the remarkable success of FeLV vaccination and management programmes in reducing FeLV prevalence globally, the incidence of feline lymphoma has not declined proportionally (Louwerens et al., 2005; Vail et al., 2019). In the 1970s, approximately 70–80% of feline lymphoma cases were attributed to FeLV (Cotter et al., 1975), whereas current data indicate that fewer than 15–25% of lymphoma cases are FeLV-associated (Louwerens et al., 2005; Rolph and Cavanaugh, 2022). This epidemiological shift has prompted renewed interest in FIV-associated oncogenesis, the role of regressive FeLV infections in tumour development, and the potential contribution of novel infectious and non-infectious aetiological factors. This review aims to synthesise the current understanding of FeLV- and FIV-associated neoplasia, covering molecular mechanisms of oncogenesis, tumour types, epidemiological trends, and clinical implications.

Feline Leukaemia Virus (FeLV)

Virology and Infection Outcomes

FeLV is an enveloped, single-stranded RNA gammaretrovirus belonging to the family Retroviridae. Following infection, cats may develop one of three outcomes: abortive infection (complete viral clearance), regressive infection (proviral DNA persists in the genome but viraemia resolves), or progressive infection (persistent antigenemia and viraemia) (Hartmann, 2012; Hofmann-Lehmann et al., 2023). Progressively infected cats are at the highest risk of developing FeLV-associated diseases, including lymphoma, leukaemia, bone marrow suppression syndromes, and secondary infections. However, regressively infected cats, despite testing negative on routinely used antigen-based tests, retain proviral DNA integrated into their genome and may also develop lymphoma, albeit at a lower frequency (Hofmann-Lehmann et al., 2023; Hartmann, 2012). This finding has significant clinical implications, as regressive infections may be underdiagnosed using standard screening methods.

FeLV Subgroups and Pathogenesis

FeLV is classified into four subgroups (A, B, C, and T) based on envelope glycoprotein properties and receptor specificity. FeLV-A is the transmissible subgroup present in all naturally infected cats and is weakly pathogenic alone. FeLV-B arises from recombination between FeLV-A and endogenous FeLV (enFeLV) sequences in the host genome and is strongly associated with lymphoma and other lymphoproliferative diseases (Neil and Onions, 1985; Stewart et al., 2013). FeLV-C develops through mutation of the FeLV-A envelope gene and causes pure red cell aplasia. FeLV-T, a variant with tropism for T lymphocytes, causes immunodeficiency through T-cell depletion (Hartmann, 2012). The emergence of these subgroup variants from FeLV-A is a critical step in disease progression, and the presence of FeLV-B in particular is strongly correlated with neoplastic transformation.

Mechanisms of FeLV-Induced Oncogenesis

Insertional Mutagenesis

The primary mechanism by which FeLV induces neoplastic transformation is insertional mutagenesis. FeLV proviral DNA integrates into the host cell genome, and when integration occurs in proximity to cellular proto-oncogenes, it can lead to their transcriptional activation. Six common integration sites have been identified in FeLV-associated feline lymphomas: c-myc, flvi-1, flvi-2 (containing bmi-1), fit-1, pim-1, and flit-1 (Fujino et al., 2009). Activation of c-myc, a well-characterised proto-oncogene involved in cell proliferation, is one of the best-studied consequences of FeLV insertional mutagenesis. The pim-1 and bmi-1 genes are known myc-collaborators in lymphomagenesis, highlighting the cooperative nature of oncogene activation in FeLV-associated tumours (Neil and Onions, 1985; Fujino et al., 2009).

Transduction of Cellular Oncogenes — Feline Sarcoma Virus

In a process distinct from insertional mutagenesis, FeLV can capture cellular oncogenes through genetic recombination, generating acutely transforming feline sarcoma viruses (FeSV). FeSV acquires one of several oncogenes, such as v-fes, v-fms, v-fgr, or v-kit, leading to polyclonal, multicentric fibrosarcomas that typically occur in young cats after a short incubation period (Hartmann, 2012). FeSV is replication-defective and requires co-infection with FeLV-A as a helper virus. With the decline in FeLV prevalence, FeSV-associated fibrosarcomas have become correspondingly rare.

The Epidemiological Shift: Declining FeLV, Persistent Lymphoma

One of the most significant epidemiological observations in feline oncology is the paradoxical persistence — or even increase — in feline lymphoma incidence despite the dramatic decline in FeLV prevalence following the introduction of widespread vaccination and management programmes (Louwerens et al., 2005; Rolph and Cavanaugh, 2022). In the 1970s, up to 70–80% of feline lymphomas were FeLV-positive (Cotter et al., 1975); by 2005, fewer than 15% of lymphoma cases at referral institutions were FeLV-associated (Louwerens et al., 2005). Concurrently, the anatomical distribution of feline lymphoma has shifted from predominantly mediastinal and multicentric forms (typically FeLV-associated) to gastrointestinal (alimentary) lymphoma, which is usually FeLV-negative and occurs in older cats (Vail et al., 2019).

A recent study from the Netherlands by Sanchez Salguero et al. (2023) evaluated 174 cats with large cell lymphoma over a 10-year period and found that only 1 of 90 screened cats was FeLV-positive and 3 were FIV-positive, confirming the diminished role of retroviruses in feline lymphoma in regions with low retroviral prevalence. The study also demonstrated a shift toward older age at diagnosis (median 8.7 years) and predominance of alimentary and nasopharyngeal subtypes, consistent with the broader epidemiological trend. These findings suggest that non-retroviral aetiological factors — including chronic intestinal inflammation (inflammatory bowel disease), environmental tobacco smoke exposure, and possibly other infectious agents such as Helicobacter species — may play increasingly important roles in feline lymphomagenesis (Rolph and Cavanaugh, 2022; Bertone et al., 2002).

Regressive FeLV Infection and Occult Oncogenesis

An important emerging concept is the potential role of regressive (latent) FeLV infections in tumour development. Regressively infected cats harbour FeLV proviral DNA integrated into their genome despite the absence of detectable viraemia or antigenemia on standard diagnostic tests. Hofmann-Lehmann et al. (2023) emphasised that the clinical significance of regressive FeLV infections may be underestimated, as proviral DNA can be reactivated under immunosuppressive conditions, and insertional mutagenesis can occur regardless of active viral replication. Hartmann (2012) noted that regressively infected cats can develop FeLV-associated diseases including lymphoma, underscoring the need for proviral PCR-based testing in cats with unexplained lymphoproliferative disease, even when standard antigen tests are negative.

Long Terminal Repeat (LTR) Determinants

The FeLV long terminal repeat (LTR) region contains transcriptional regulatory elements that influence both viral tropism and oncogenic potential. Studies of FeLV isolates from naturally occurring lymphomas have demonstrated that specific LTR structures are associated with distinct tumour types: thymic lymphoma-derived LTRs contain transcription factor binding sites adapted for T-cell recognition, while non-T-cell disease-associated LTRs contain unique sequence motifs

(Stewart et al., 2013). These LTR variations serve as genetic determinants of tumour phenotype and contribute to the diversity of FeLV-associated neoplasms.

Tumour Types Associated with FeLV

Lymphoma is the most common neoplasm associated with FeLV infection and was historically the most frequent malignancy in domestic cats overall (Vail et al., 2019). FeLV-associated lymphomas are predominantly of T-cell origin and most commonly present as mediastinal (thymic) or multicentric forms, particularly in young cats (Vail et al., 2019; Cotter et al., 1975). With the decline in FeLV prevalence, the anatomical distribution of feline lymphoma has shifted, with gastrointestinal (alimentary) lymphoma — which is predominantly FeLV-negative — becoming the most common form in many populations (Louwerens et al., 2005; Rolph and Cavanaugh, 2022).

Beyond lymphoma, FeLV is associated with various forms of leukaemia, including lymphoid, myeloid, erythroid, and megakaryocytic leukaemias (Hartmann, 2012). Myeloproliferative diseases and myelodysplastic syndrome (MDS) are also well-documented complications of progressive FeLV infection. FeSV-induced multicentric fibrosarcomas, although rare today, represent another important FeLV-associated neoplasm. Hodgkin's-like lymphoma, olfactory neuroblastoma, and osteochondromatosis have also been sporadically associated with FeLV infection (Hartmann, 2012; Rolph and Cavanaugh, 2022).

Feline Immunodeficiency Virus (FIV)

Virology and Pathogenesis

FIV, first isolated by Pedersen et al. (1987) from a group of cats in Petaluma, California, is a lentivirus classified within the family Retroviridae. FIV infection follows a clinical course analogous to HIV infection in humans, progressing through acute, asymptomatic, and terminal (AIDS-like) phases characterised by progressive CD4+ T lymphocyte depletion, immunodeficiency, and susceptibility to opportunistic infections and neoplasia (Hartmann, 2012; Bęczkowski et al., 2025). FIV prevalence in healthy cats is approximately 2–4% in most countries, while in sick or high-risk cats it may reach 15–30% (Hartmann, 2012). Risk factors for FIV infection include male sex, outdoor access, and aggressive behaviour (bite wounds being the primary route of transmission).

FIV and Neoplasia

The association between FIV infection and neoplasia, particularly lymphoma, has been consistently demonstrated in epidemiological studies. Shelton et al. (1990) identified a significant independent correlation between FIV infection and lymphoproliferative malignancies. Louwerens et al. (2005) quantified the risk, reporting a 5.6-fold increased likelihood of lymphoma development in FIV-positive cats. In one Australian cohort study, lymphoma was the most common condition diagnosed at post-mortem examination, affecting 56% of FIV-infected cats that died (Bęczkowski et al., 2014). The prevalence of FIV infection in one cohort of cats with lymphoma was 50%, substantially higher than the FIV prevalence in the general population, providing further support for a causal relationship (Gabor et al., 2001). In a survey of 826 naturally FIV-infected cats examined at North American Veterinary Teaching Hospitals, neoplasia — especially lymphoma and cutaneous squamous cell carcinoma — was among the most common disease syndromes (Hartmann, 2012).

Mechanisms of FIV-Associated Oncogenesis

Indirect Mechanisms: Immune Dysregulation

The predominant mechanism by which FIV promotes tumourigenesis is considered indirect, involving chronic immune dysregulation and impaired tumour immunosurveillance. Progressive CD4+ T-cell depletion leads to a diminished capacity for immune-mediated elimination of transformed cells. Concurrently, chronic antigenic stimulation of B lymphocytes by persistent viral replication promotes polyclonal B-cell activation and hyperactivity, creating a permissive environment for the emergence of clonal malignancies (Callanan et al., 1996; Beatty et al., 1998). This mechanism parallels the pathogenesis of HIV-associated lymphomas in humans, where chronic immune activation and B-cell stimulation are well-established oncogenic factors.

Callanan et al. (1996) characterised the immunological deficits in FIV-infected cats that developed lymphoma and identified impaired cell-mediated immunity combined with B-cell hyperactivity as potential indirect mechanisms in FIV-associated lymphomagenesis. FIV-associated lymphomas are predominantly high-grade B-cell neoplasms of the centroblastic or immunoblastic subtypes, frequently presenting at extranodal sites — features that closely mirror HIV-associated lymphomas in humans (Callanan et al., 1996;

Beatty et al., 1998).

Direct Mechanisms: Insertional Mutagenesis

Although FIV is primarily considered an indirect oncogenic agent, evidence for occasional direct insertional oncogenesis exists. Beatty et al. (1998) demonstrated clonally integrated FIV proviral DNA in lymphoma cells from an experimentally infected cat, providing the first evidence that FIV could directly contribute to malignant transformation through insertional mutagenesis in rare cases. This finding suggests that while indirect immune-mediated mechanisms predominate, FIV may occasionally act as a direct oncogene, particularly in cases of long-standing infection (Beatty et al., 1998; Hartmann, 2012).

Tumour Types Associated with FIV

Lymphoma is the most common neoplasm associated with FIV infection. Unlike FeLV-associated lymphomas, which are predominantly T-cell-derived and mediastinal, FIV-associated lymphomas are most commonly B-cell-origin, high-grade, and arise at extranodal sites including the kidneys, liver, central nervous system, and nasal cavity (Callanan et al., 1996; Beatty et al., 1998; Hartmann, 2012). Myeloproliferative disease and cutaneous squamous cell carcinoma have also been reported in association with FIV infection. Shelton et al. (1990) described two co-infected (FIV+FeLV+FeSV) cats with multicentric fibrosarcomas. Although squamous cell carcinoma has been observed in FIV-infected cats, the causal relationship remains less clearly defined, as this tumour is common in older cats regardless of retroviral status and may involve feline papillomavirus co-infection (Rolph and Cavanaugh, 2022).

FeLV-FIV Co-infection and Synergistic Oncogenic Risk

Co-infection with FeLV and FIV represents a particularly high-risk scenario for neoplastic disease. Louwerens et al. (2005) demonstrated that the risk of lymphoma in co-infected cats was increased 77-fold compared to uninfected controls — far exceeding the additive risk of either infection alone. Pedersen et al. (1990) demonstrated experimentally that pre-existing FeLV infection greatly potentiated the severity of FIV infection, with enhanced FIV proviral DNA expression in both lymphoid and non-lymphoid tissues and more severe CD4+/CD8+ ratio inversion. Shelton et al. (1990) reported that illnesses in co-infected cats were similar to those of cats infected with FeLV alone, suggesting that FeLV-associated pathogenesis may dominate in the dual-infection context, but with accelerated immunodeficiency contributed by FIV.

The synergistic mechanisms likely involve: (1) FIV-mediated acceleration of CD4+ T-cell depletion, weakening antiviral and antitumour immunity; (2) FeLV-mediated insertional mutagenesis operating in a permissive immunological environment created by FIV; and (3) enhanced replication and dissemination of both viruses within shared target cell populations. Clinically, co-infected cats present earlier, with more severe disease manifestations and shorter survival times than cats infected with either virus alone (Pedersen et al., 1990).

Clinical Implications and Future Perspectives

The recognition of retroviral oncogenesis in cats has profound implications for clinical practice. Routine screening of all cats for FeLV and FIV is recommended, particularly in animals presenting with neoplasia. The 2020 American Association of Feline Practitioners (AAFP) retrovirus management guidelines advocate for universal testing, identification of retroviral status prior to vaccination, and appropriate isolation or management of infected individuals (Little et al., 2020). FeLV vaccination remains the cornerstone of prevention; however, awareness of the continued oncogenic potential of regressive FeLV infections and FIV-associated neoplasia is essential for comprehensive clinical management.

The prognosis for cats with retrovirus-associated lymphoma is generally poorer than for retrovirus-negative cats. FeLV-positive cats with lymphoma exhibit shorter remission and survival times following chemotherapy, partly due to concurrent immunosuppression and increased susceptibility to secondary infections during treatment (Vail et al., 2019). Nevertheless, even progressively FeLV-infected cats with lymphoma can respond favourably to chemotherapy, and treatment should be offered when appropriate.

Future research directions include the development of more sensitive diagnostic tools for detecting regressive FeLV infections, further elucidation of FIV-associated oncogenic mechanisms at the molecular level, investigation of the potential oncogenic role of endogenous FeLV sequences, exploration of novel infectious agents (e.g., feline papillomaviruses, Helicobacter spp.) in

feline tumourigenesis, and the development of immunotherapeutic strategies targeting virus-associated tumour antigens. The feline model continues to provide valuable comparative insights into human retroviral oncology, with FIV-associated lymphoma serving as a natural analogue of HIV-associated non-Hodgkin lymphoma (Beatty et al., 1998; Callanan et al., 1996).

Conclusion

FeLV and FIV remain the most clinically significant oncogenic agents in domestic cats, despite the declining prevalence of FeLV in many populations. FeLV-induced oncogenesis, operating through insertional mutagenesis, proto-oncogene transduction, and LTR-mediated transcriptional activation, represents one of the best-characterised models of retroviral tumourigenesis in veterinary medicine. FIV-associated oncogenesis, predominantly driven by chronic immune dysregulation and impaired tumour immunosurveillance, parallels the mechanisms observed in HIV-associated malignancies in humans. The synergistic oncogenic risk of co-infection underscores the importance of screening for both viruses in feline patients.

The epidemiological shift from predominantly FeLV-associated mediastinal lymphoma to FeLV-negative gastrointestinal lymphoma in ageing cat populations highlights the evolving landscape of feline oncology and suggests that non-retroviral aetiological factors require further investigation. Comprehensive retroviral testing, appropriate vaccination strategies, and awareness of the oncogenic potential of both progressive and regressive infections are essential components of feline healthcare. Continued research into the molecular mechanisms of retroviral oncogenesis will not only benefit feline patients but also contribute to the broader understanding of virus-associated cancers across species.

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Research Article

An Investigation of Certain Inflammatory Markers in Whole Blood in Patients with Feline Panleukopenia

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Abstract

Feline Panleukopenia Virus (FPV) is a highly contagious viral infection, most commonly observed in young and unvaccinated cats, characterized by high morbidity and mortality rates. Although several diagnostic methods exist, recent studies have increasingly emphasized the use of inflammation indicators derived from complete blood count (CBC) in the diagnosis and management of FPV. In the present study, CBC results from 25 infected cats (older than 45 days, regardless of sex) presenting to a veterinary hospital with complaints of vomiting and diarrhea, along with 10 healthy cats, were evaluated. Derived hematological indices including Neutrophil-to-Lymphocyte Ratio (NLR), Lymphocyte-to-Monocyte Ratio (LMR), Platelet-to-Lymphocyte Ratio (PLR), Systemic Immune-Inflammation Index (SII), Systemic Inflammatory Response Index (SIRI), Pan-Immune Inflammation Value (PIV), and Neutrophil-Monocyte-Lymphocyte Ratio (NMLR) were analyzed. The study demonstrated statistically significant differences in NLR ($p = 0.008$), PLR ($p = 0.019$), NMLR ($p = 0.007$), SII ($p < 0.001$), SIRI ($p = 0.001$), and PIV ($p < 0.001$). These findings suggest that such parameters may serve as valuable tools in the diagnosis, clinical management, and prognostic assessment of FPV. It is concluded that with further comprehensive studies, the clinical utility of these indices will gain increasing importance.
Keywords: FPV, Inflammation Index, Whole Blood, NLR, SIRI

Introduction

Feline panleukopenia virus (FPV) is a non-enveloped, single-stranded DNA parvovirus that is widely distributed among domestic and wild felids worldwide (Neuerer et al., 2008; Sykes & Parrish, 2021). The virus can also infect certain carnivores, such as foxes and raccoons, as well as some primate species (Scott, 1987). FPV possesses a ~5 kb genome with terminal hairpin structures that are essential for viral replication and transcription (Cotmore & Tattersal, 2013), 2013). Clinically, infection is characterized by severe leukopenia and acute enteritis, particularly in kittens and unvaccinated cats (Pedersen, 1988; Decaro & Buonavoglia, 2012). Animal shelters and other high-density environments facilitate viral transmission and have been associated with significant morbidity and mortality during outbreaks (Addie et al., 1998).

Experimental studies have demonstrated that FPV can hemagglutinate erythrocytes from various species and infect a broad range of hosts under laboratory conditions; however, this does not necessarily indicate natural susceptibility (Decaro & Buonavoglia, 2012; Tuzio, 2021). FPV is closely related to canine parvovirus type 2 (CPV-2), which emerged in the 1970s and subsequently evolved into the CPV-2a, CPV-2b, and CPV-2c variants (Nakamura et al., 2001). The genetic and antigenic similarity between FPV and CPV-2 has facilitated cross-species adaptation, enabling certain CPV variants to infect cats (Decaro & Buonavoglia, 2012).

In addition to causing bone marrow suppression and gastrointestinal damage, FPV infection induces

significant hematological alterations that may aid in disease assessment. Recently, inflammatory biomarkers derived from complete blood counts, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR), have gained attention as indicators of disease severity and prognosis (Rejec et al., 2017). These markers are associated with characteristic hematological abnormalities such as leukopenia and lymphopenia and offer a rapid, cost-effective approach for risk assessment and outbreak management, particularly in shelter populations (Desai et al., 2021; Yanar, 2024).

Materials and Methods

Study Cohort

The study population consisted of 25 cats with feline panleukopenia and 10 clinically healthy controls, all older than 45 days of age and of either sex, that were presented to the Animal Hospital of the Faculty of Veterinary Medicine, Kırıkkale University.

Clinical Examination Protocol

Cats admitted to the Internal Medicine Clinic of the Animal Hospital, Faculty of Veterinary Medicine, Kırıkkale University, with a history of vomiting and diarrhea were enrolled in the study. Information regarding age, breed, sex, and vaccination status was recorded, and a complete physical examination was performed. Vital parameters were documented, and cats were subsequently evaluated as suspected feline panleukopenia cases. Definitive diagnosis was established using additional diagnostic methods to determine the underlying etiology.

The control group comprised clinically healthy cats presented for routine vaccination and health screening. Complete blood count, serum biochemical parameters, and physical examination findings were obtained and recorded for all control animals.

Infected Group

A total of 25 cats with feline panleukopenia were included in the study. The infected group was composed of cats exhibiting clinical signs compatible with feline panleukopenia, including vomiting, diarrhea, depression, and anorexia. Following clinical assessment, blood samples were collected and subjected to complete blood count analysis. Cats demonstrating leukopenia, as evidenced by total leukocyte (WBC) counts below the reference range, were further evaluated using a lateral flow immunochromatographic rapid antigen test for feline panleukopenia virus (FPV). Cats testing positive were considered infected and subsequently included in the study group.

Control Group

Ten clinically healthy cats presented for routine vaccination and general health assessment were enrolled as the control group. Inclusion criteria for the control group consisted of the absence of clinical signs of disease and hematological and biochemical parameters within reference ranges, as determined through routine health screening examinations performed at the Animal Hospital of Kırıkkale University Faculty of Veterinary Medicine.

Collecting Samples and Laboratory Analyses

For hematological assessment, 2 mL of blood was collected from the cephalic vein (vena cephalica antebrachii) of each cat into EDTA-containing tubes. Samples were analyzed within 30 minutes of collection using an automated hematology analyzer (Mindray BC-5000, China) to obtain complete blood count (CBC) parameters. The resulting hematological data were recorded for subsequent statistical analysis.

Application of Rapid Tests

Rapid diagnostic test kits are widely recognized as a valid diagnostic tool for the detection of feline panleukopenia virus (FPV) infection. As FPV is shed through the gastrointestinal tract and is associated with prominent enteric manifestations, fecal or rectal swab samples were collected from all cats in both the infected and control groups for the detection of FPV antigen. For fecal samples, the collection swab provided with the test kit was used to obtain the required amount of fecal material. In cases where rectal swab samples were collected, the procedure was initiated directly using the swab specimen. The fecal sample or rectal swab was thoroughly mixed with the dilution buffer supplied in the test kit to ensure adequate sample homogenization. The resulting suspension was allowed to stand on a flat surface for sedimentation of coarse particulate matter. Subsequently, 10 µL (approximately three drops) of the clarified diluted sample was transferred using the provided dropper and dispensed into the sample well of the test cassette positioned on a level surface. Test results were interpreted after an incubation period of 8 minutes according to the manufacturer's instructions (VetExpert FPV Ag Test, Poland). The presence of a control line was required to validate the test. Samples yielding invalid results, indicated by the absence of a control line, were excluded from the study.

The appearance of a test line was interpreted as a positive result for FPV antigen detection, regardless of the intensity or clarity of the line. Test interpretation and result classification were performed strictly in accordance with the manufacturer's guidelines, with the presence of the test line serving as the sole criterion for a positive result.

Calculating the Inflammation Indıcators

In the present study, seven inflammatory biomarkers were evaluated. The calculated inflammatory indices were derived from the complete blood count (CBC) data obtained from each animal. Hematological inflammatory indices were calculated using formulas previously described in the literature. The evaluated parameters and the corresponding formulas used for their calculation are presented below;

- Notrophile Lymphosit Ratio (NLR) = Notrophile Count/Lymphosite Count

- Lymphosite Monocyte Ratio (LMR) = Lymphosite Count/ Monocyte Count
- Platelete Lymphosite Ratio (PLR) = Platelete Count/Lymphiste Count
- Systemic Inflamatoric Immune Index (SII) = Trombocyte Count X Notrophile Count/Lymposite Count
- Systemic Inflamatoric Response Index (SIRI) = Notrophile Count X Monocyte Count/Lymphosite Count
- Pan Immune Inflamatoric Value (PIV) = Nortophile Count X Monocyte Count X Trombocyte Count/Lymphosite Count
- Notrophile Monocyte Lymphosite Raito = Monocyte Count + Notrophile Count / Lymphosite Count

Statistical Analyses

The Shapiro-Wilk test was used to assess the normality of the data distribution across groups. Mean and standard deviation values were reported for normally distributed data, while median and interquartile range (IQR) values were reported for non-normally distributed data. The Student's t-test was used to compare data with a normal distribution, while the Mann-Whitney U test was applied to data with a non-normal distribution. A p-value of <0.05 was considered a statistically significant difference. The Jamovi Project 2.6 (Australia) was used to perform the statistical analyses.

Results

The study evaluated complete blood count results obtained from the control and infected groups. The leukogram findings and statistical analyses of the complete blood count are presented in Table 1. When the leukogram findings across the study groups were evaluated, it was observed that the total white blood cell (WBC) counts of cats infected with panleukopenia were below the reference ranges. Leukopenia was detected in all infected animals. It was determined that there was a statistically significant difference between the WBC counts of the control group and the infected group ($p<0.05$). Similarly, it was observed that the counts of neutrophils (NEU), lymphocytes (LEU), monocytes (MON), eosinophils (EOS), and basophils (BAS) were lower than those in the control group and showed statistically significant differences ($p<0.001$, $p<0.05$).

Table 1. Results of Haemogram

	CONTROL		INFECTED		
	Mean (Min-Max)	Median (IQR)	Mean (Min-Max)	Median (IQR)	P Değeri
WBC	10.6 (5.50-16.3)	9.52 (4.80)	1.82 (0.26-4.78)	1.24 (2.16)	**<0.001**
NEU	7.19 (3.51-12.54)	7.21 (2.33)	0.92 (0.05-3.35)	0.71 (0.84)	**<0.001**
LYM	2.78 (0.92-6.05)	2.34 (1.41)	0.67 (0.11-2.24)	0.29 (0.83)	**<0.001**
MON	0.17 (0.08-0.40)	0.13 (0.08)	0.09 (0.00-0.94)	0.05 (0.06)	**<0.001**
EOS	0.39 (0.21-0.76)	0.32 (0.14)	0.12 (0.00-0.55)	0.03 (0.22)	**<0.001**
BAS	0.02 (0.00-0.10)	0.01 (0.02)	0.00 (0.00-0.06)	0.00 (0.00)	**0.002**

The study evaluated the results of complete blood count parameters for the control and infected groups in light of parameters reported in the literature. The first parameter examined was the neutrophil-to-lymphocyte ratio (NLR). It was found that the mean value in the infected group was lower than that in the control group, and a similar result was observed for the median value. In the intergroup statistical analysis, a significant statistical difference was observed between the control group and the infected group (P=0.008). A similar situation was observed for the platelet-to-lymphocyte ratio. The mean and median values for the infected group were lower, and a statistically significant difference between the groups was detected (P=0.019). The situation was different for the lymphocyte-to-monocyte ratio. The mean for the infected group was found to be higher than that of the control group, and no statistically significant difference was found between the groups (P=0.315). For SII and SIRI values, the mean values in the infected group were found to be higher than those in the control group. A statistically significant difference was detected between the groups for SII and SIRI values ($p<0.001$, P=0.001). For the remaining PIV and NMLR values, excluding the 5 parameters, the differences between the groups were found to be statistically significant ($P<0.001$, P=0.007), and the mean values of the infected group were lower than those of the control group. The mean, median, and statistical results for the hematological index parameters are shown in Table 2

Table 2. Results of Haematological Index

	CONTROL		INFECTED		
	Mean (Min-Max)	Median (IQR)	Mean (Min-Max)	Median (IQR)	P Value
NLR	3.02 (1.32-4.77)	2.77 (1.95)	2.46 (0.23-19.7)	1.29 (1.14)	**0.008**
LMR	17.2 (7.62-28.8)	13.9 (10.2)	17.9 (0.00-112)	9.67 (20.7)	0.315
PLR	125 (40.0-295)	93.2 (108)	333 (64.7-1135)	192 (131)	**0.019**
SII	751 (427-1261)	625 (461)	183 (14.0-636)	134 (156)	**<0.001**

SIRI	0.53 (0.19-1.62)	0.41 (0.26)	0.39 (0.00-6.80)	0.04 (0.17)	**0.001**
PIV	122 (57.0-261)	106 (92.7)	23.7 (0.00-340)	6.88 (8.23)	**<0.001**
NMLR	3.20 (1.53-5.11)	3.01 (1.97)	2.56 (0.24-19.7)	1.35 (1.37)	**0.007**

Discussion

This study evaluated seven hematological inflammatory indices derived from complete blood count parameters in cats with feline panleukopenia virus (FPV) infection. Consistent with previous reports, FPV-infected cats exhibited marked leukopenia, with all infected animals demonstrating WBC counts below the reference range (Tuzio, 2021). Significant reductions in neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts were also observed, reflecting the well-established tropism of FPV for rapidly dividing cells within the bone marrow and lymphoid tissues (Truyen et al., 2009; J. E. Sykes & Parrish, 2021). These findings further support leukopenia as a hallmark hematological feature of FPV infection.

Although anemia has frequently been reported in FPV-infected cats (Kadam et al., 2022; Modi et al., 2024), no significant differences in RBC, HGB, or HCT values were detected between groups. Nevertheless, infected cats exhibited numerically lower mean erythrocyte-related values than healthy controls. Previous studies suggest that anemia in FPV may depend on disease stage, severity of bone marrow suppression, hydration status, and concurrent complications (Truyen et al., 2009; Gülersoy et al., 2023). Similarly, erythrocyte indices (MCV, MCH, and MCHC) did not differ significantly between groups, in agreement with most published reports describing FPV-associated anemia as predominantly normocytic and normochromic (Kadam et al., 2022; Gülersoy et al., 2023; Modi et al., 2024).

Among the inflammatory indices evaluated, NLR was significantly lower in FPV-infected cats. This finding is consistent with previous studies and likely reflects the profound neutropenia caused by FPV-induced bone marrow suppression (Yanar, 2024; Aydin, 2025). In contrast to many inflammatory disorders where NLR increases, the severe cytopenic nature of FPV leads to a marked reduction in circulating neutrophils, making low NLR a potentially useful indicator of disease severity and prognosis.

LMR did not differ significantly between groups, despite a higher mean value in infected cats. Similar findings have been reported previously (Aydin, 2025). The absence of a significant difference may be explained by the simultaneous depletion of both lymphocytes and monocytes in FPV infection, resulting in relative preservation of their ratio (Tuna & Kirkulak, 2023). However, the limited number of studies investigating LMR in feline panleukopenia warrants further research.

PLR was significantly elevated in infected cats, supporting previous observations in FPV cases (Donato et al., 2023; Yanar, 2024; Aydin, 2025). This increase appears to be primarily driven by severe lymphopenia rather than thrombocytosis. Given its accessibility and low cost, PLR may serve as a useful adjunctive biomarker for assessing inflammatory burden and prognosis in cats with FPV.

Both SII and SIRI were significantly lower in infected cats. While these indices are often elevated in inflammatory diseases, FPV represents a unique condition characterized by profound immunosuppression and pancytopenia (Xia et al., 2023; Aydin, 2025). Consequently, reductions in neutrophil and platelet counts appear to outweigh inflammatory responses, resulting in lower SII and SIRI values. These findings suggest that both indices may provide useful information regarding the degree of immune suppression associated with FPV infection.

To our knowledge, this is among the first studies evaluating PIV in cats with FPV. PIV values were significantly lower in infected animals, likely reflecting the combined effects of neutropenia, monocytopenia, lymphopenia, and altered platelet dynamics. Although extensively investigated in human medicine as a prognostic inflammatory biomarker (Guven et al., 2022; Kuang et al., 2024), its role in feline infectious diseases remains largely unexplored. The present results indicate that PIV may have potential clinical utility in FPV and deserves further investigation.

Similarly, NMLR was significantly reduced in the infected group. Given the severe alterations in neutrophil, monocyte, and lymphocyte populations associated with FPV infection, this index appears capable of reflecting disease-associated systemic immune dysregulation. Although evidence regarding NMLR in veterinary medicine remains limited, the significant findings obtained in this

study suggest that it may represent a practical and cost-effective prognostic biomarker for cats with FPV.

Overall, the present findings indicate that NLR, PLR, SII, SIRI, PIV, and NMLR are significantly altered in cats with feline panleukopenia and may provide valuable adjunctive information for disease assessment and prognostic evaluation. Among these indices, NLR, SII, SIRI, PIV, and NMLR appear particularly promising because they reflect the characteristic cytopenic and immunosuppressive pathophysiology of FPV infection. Further studies involving larger populations and outcome-based analyses are required to establish their prognostic value in clinical practice.

Ethical Approval

This study was approved ethical status by the Kırıkkale University Local Ethics Committee on Animal Experiments (Decision Number: 2025/01/07)

Conflict of Interest

The outhors declared that there is no conflict of interest.

Authors' Contributions

This research was designed by BYG and YP. In Data collection adn processing part was made by BYG and YP. Analysis were made by YP. Literature review protocols were realised by BYG and YP. The article was written by BYG and YP.

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Research Article

Assessment of NT proBNP, Feline Pancreatic Lipase and Neutrophil/Lymphocyte Ratio in Cats

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Abstract

Cardiovascular diseases and pancreatitis represent significant clinical entities in veterinary medicine, both as primary conditions and through their mutual influence on each other's pathophysiological processes. While numerous studies in human medicine have demonstrated the relationship between cardiac injury and pancreatitis, investigations addressing this interaction in cats remain limited. The primary objective of this study was to evaluate the relationship among N-terminal pro-B-type natriuretic peptide (NT-proBNP), a biomarker of cardiac injury; feline pancreatic lipase (fPL), a diagnostic marker of pancreatitis; and the neutrophil-to-lymphocyte ratio (NLR), an indicator of systemic inflammation, independent of disease status in cats. The study was conducted on 20 cats of various breeds, ages (mean 4.75 years), and sexes presented to the Veterinary Teaching Hospital of Kırıkkale University. Blood samples were collected to measure NT-proBNP and fPL concentrations, while complete blood count data were used to calculate NLR values. Statistical analyses included the Shapiro–Wilk test for normality, Spearman's correlation analysis to assess relationships among parameters. The analyses revealed no statistically significant associations among the biomarkers examined. Correlation analysis demonstrated very weak and non-significant relationships ($p > 0.05$) between NT-proBNP and fPL ($r = 0.067$, $p = 0.779$), NT-proBNP and NLR ($r = 0.263$, $p = 0.263$), and fPL and NLR ($r = 0.274$, $p = 0.242$). The findings suggest that NT-proBNP, fPL, and NLR alone are not sufficient to explain the coexistence of pancreatitis in cats with cardiomyopathy or suspected cardiac injury. Although these biomarkers provide valuable clinical information, definitive diagnosis requires integration with physical examination, medical history, and imaging modalities such as ultrasonography. Furthermore, the complexity of the underlying mechanisms highlights the need for larger-scale, prospective studies to achieve a more comprehensive understanding of this interplay.

Keywords: Cat, fPL, NT-proBNP, NLR, veterinary

Introductıon

Cardiovascular diseases are among the most important health concerns in both human and veterinary medicine. In cats, advances in genetic research and increased access to echocardiographic examinations have improved the recognition and diagnosis of cardiac disorders, particularly in predisposed breeds (Egenvall et al., 2009; Fox et al., 2019). Several biomarkers, including cardiac troponins, N-terminal pro-atrial natriuretic peptide (NT-proANP), and N-terminal pro-B-type natriuretic peptide (NT-proBNP), are routinely used to assess myocardial injury and cardiac dysfunction (Borgeat et al., 2018).

NT-proBNP is a biologically inactive peptide released from cardiomyocytes in response to myocardial stress and damage, regardless of the underlying cause (Hall, 2004; Ferasin, 2012). In

both human and veterinary medicine, NT-proBNP has been widely investigated as a diagnostic and prognostic biomarker for cardiovascular disease, particularly cardiomyopathies and heart failure (Maron et al., 2006; Borgeat et al., 2018; Luis Fuentes et al., 2020).

Pancreatitis is a common inflammatory disease in cats and may occur in acute or chronic forms. Acute pancreatitis is characterized by premature activation of pancreatic enzymes resulting in pancreatic autodigestion and systemic inflammatory responses, whereas chronic pancreatitis is associated with progressive fibrosis and structural pancreatic damage (Pham & Forsmark, 2018; Mederos et al., 2021). Previous studies have reported a relationship between pancreatitis and cardiovascular disorders, suggesting that systemic inflammation may contribute to myocardial injury and cardiovascular complications (Bexelius et al., 2013; Chung & Lin, 2017; Chen et al., 2023).

The neutrophil-to-lymphocyte ratio (NLR) is an inexpensive and readily available indicator of systemic inflammation and physiological stress. Increasing evidence suggests that NLR may have diagnostic and prognostic value in both cardiovascular diseases and pancreatitis in humans and animals (Yurtdas et al., 2018; Fries et al., 2022; Esin & Uzun, 2025). However, information regarding the relationship between pancreatic disease, cardiac biomarkers, and inflammatory indices in cats remains limited.

Therefore, the aim of the present study was to evaluate circulating NT-proBNP concentrations and neutrophil-to-lymphocyte ratio values in cats with pancreatitis and to investigate their potential clinical significance in assessing cardiac involvement associated with pancreatic inflammation.

Materials and Methods

Study Cohort

This study was conducted using 20 client-owned cats presented to the Kırıkkale University Veterinary Teaching Hospital. Cats of any breed and sex aged ≥6 months were eligible for inclusion. Plasma NT-proBNP concentrations, feline pancreatic lipase (fPL) results, and neutrophil-to-lymphocyte ratio (NLR) values were retrospectively obtained from routine clinical and laboratory evaluations, irrespective of the presenting complaint or clinical diagnosis. These parameters were subsequently included in the study analysis.

Demographic data, including age, sex, and breed, were recorded for all cats. Although demographic characteristics were documented, no subgroup analyses or classifications were performed based on these variables due to the sample size. The study design and organization were developed with reference to previously published studies in human medicine and the limited available veterinary literature investigating similar associations between pancreatic disease, inflammatory markers, and cardiac biomarkers.

Clinical Examination Protocol

Following the evaluation of demographic characteristics, cats were selected using a random sampling approach from animals presented to the Kırıkkale University Veterinary Teaching Hospital. No specific presenting complaint was established as an inclusion criterion. Healthy cats were enrolled from animals presented for routine health examinations, and inclusion was based on owner consent for the use of clinical and laboratory data.

Cats included in the diseased group were selected based on the presence of clinical signs consistent with pancreatitis or conditions with the potential to impose hemodynamic stress on the cardiovascular system, regardless of the underlying etiology. Clinical findings considered during case selection included vomiting, diarrhea, lethargy, anorexia or hyporexia, and dehydration. Healthy cats were recruited from individuals undergoing routine wellness examinations and displaying no evidence of systemic disease at the time of evaluation.

Demographic information, including age, sex, and breed, was recorded for all enrolled cats and used for descriptive purposes. No further stratification based on demographic variables was performed.

Collecting Samples and Laboratory Analyses

For the analysis of the 20 cats included in the study, blood samples were collected from the vena cephalica antebrachii under routine clinical conditions. Approximately 2.5 mL of blood was transferred into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Immediately thereafter, an additional 3 mL of blood was collected into anticoagulant-free tubes for serum separation.

To obtain plasma, EDTA blood samples were centrifuged at 3,000 rpm for 10 min. Following centrifugation, the plasma fraction was carefully aspirated using a micropipette and transferred into Eppendorf tubes. Plasma samples were stored at −20°C until analysis of feline pancreatic lipase (fPL)

and N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations.

For serum preparation, blood samples collected into anticoagulant-free tubes were allowed to clot in an upright position at room temperature for 1 h. Subsequently, the samples were centrifuged at 2,000 rpm for 15 min. The clear supernatant serum was separated using a micropipette and transferred into sterile Eppendorf tubes. Serum samples were stored at −20°C until biomarker analysis.

Haematological Analyses

Hematological parameters were determined using an automated hematology analyzer (Mindray BC-5000; Mindray Bio-Medical Electronics Co., Shenzhen, China). Blood samples collected into EDTA-containing tubes were analyzed within 30 min of collection to minimize pre-analytical variation. The hematological results obtained from the analyzer were recorded and subsequently used for study analyses. The neutrophil-to-lymphocyte ratio (NLR) was calculated from the absolute neutrophil and lymphocyte counts derived from the complete blood count results.

Biomarker Analyses

Plasma and serum samples obtained from the enrolled cats were used for the measurement of NT-proBNP and feline pancreatic lipase (fPL) concentrations. Following separation, samples were aliquoted and stored at −20°C until analysis. All samples were maintained under appropriate cold-chain conditions to preserve analyte stability prior to testing.

NT-proBNP and fPL analyses were performed using automated veterinary diagnostic platforms (Vcheck V200, Bionote Co., Ltd., Republic of Korea, and VetXpert Smart Cube, Mindray Animal Medical Technology Co., Ltd., China) in accordance with the manufacturers' protocols. The resulting biomarker concentrations were recorded and used for subsequent statistical evaluation.

Statistical Analyses

Statistical analyses were performed using Jamovi software (version 2.6; The Jamovi Project, Australia). As no predefined study groups were established, the relationships among the measured variables were evaluated across the entire study population. Demographic characteristics of the enrolled cats were summarized using descriptive statistical methods.

The distribution of continuous variables was assessed using the Shapiro-Wilk normality test. Associations between variables were evaluated using Spearman's rank correlation coefficient.. Statistical significance was set at $P < 0.05$.

Results

Demographic Data

The breed, age, and sex distributions of the cats included in the study are presented in Table 4.1. The mean age of the study population was 4.75 years. With respect to sex distribution, 10 cats (50%) were male and 10 cats (50%) were female. Breed distribution analysis revealed that 11 cats (55%) were domestic shorthair (tabby), 3 cats (15%) were Scottish, 3 cats (15%) were British Shorthair, 2 cats (10%) were Siamese, and 1 cat (5%) was an orange tabby (Sarman). These demographic data were recorded and included in the descriptive analysis of the study population. Due to the limited sample size, the study data were not stratified according to age, breed, or sex. Consequently, no statistical analyses were performed to evaluate the potential effects of these demographic variables on the measured parameters. Demographic characteristics were therefore reported descriptively and were not included as factors in the inferential statistical analyses. The results were shown in table 1.

Table1. Demographic Data

Age (Mounth)	Mean	4.75
	Min-Max	1-13
Sex	Erkek	10 (%50)
	Dişi	10 (%50)
Breed	Tabby	11 (%55)
	Scottish	3 (%15)
	British	3 (%15)
	Siamese	2 (%10)
	Orange Tabby	1 (%5)

NT proBNP, fPL and NLR Ratio Levels

NT-proBNP concentrations of the cats included in the study were evaluated using descriptive statistical methods. The median, mean, minimum, and maximum NT-proBNP values are presented in Table 2. As all cats were included in the analysis regardless of disease status, several NT-proBNP measurements were outside the established reference interval. Therefore, the analyses focused on assessing the relationships among the measured variables independently of reference range classifications. In this context, variations in biomarker concentrations were evaluated according to their relative increases or decreases, irrespective of the underlying disease condition.

Table 2. NT proBNP, fPL and NLR ratio levels

	Mean (Min-Max)	Median (IQR)	SD
NT proBNP (pmol/L)	341 (50-1500)	98.9 (146)	524
fPL (ng/ml)	8.28 (1-41.5)	1.98 (5.35)	13.0
NLR	11.0 (0.198-128)	3.17 (4.29)	28.3

Correlation Analyses Results

Assessment of data distribution using the Shapiro-Wilk test demonstrated that the variables were not normally distributed. Therefore, correlations between variables were evaluated using Spearman's rank correlation analysis.

The correlation coefficient between NT-proBNP and fPL concentrations was r = 0.067, indicating a very weak positive association. Furthermore, the relationship was not statistically significant (P = 0.779; P > 0.05). For NT-proBNP and NLR, the correlation coefficient was r = 0.263, suggesting a weak positive relationship. However, this association did not reach statistical significance (P = 0.263; P > 0.05). Similarly, the correlation analysis between fPL and NLR yielded a correlation coefficient of r = 0.274, indicating a weak positive association. The corresponding P-value was 0.242 (P > 0.05), demonstrating the absence of a statistically significant relationship between these variables. Given the relatively small sample size (n = 20; degrees of freedom = 18), the statistical power of the correlation analyses was limited. Consequently, the non-significant P-values suggest that no evidence of a meaningful monotonic relationship was identified among the examined variables within the study population. A summary of the Spearman correlation analysis results is presented in Table 3.

Table 3. Spearman Correlation Test Results

		NT proBNP	fPL	NLR
NT proBNP	Spearman rho	-		
	df	-		
	P value	-		
fPL	Spearman rho	0.067	-	
	df	18	-	
	P value	0.779	-	
NLR	Spearman rho	0.263	0.274	-
	df	18	18	-
	P value	0.263	0.242	-

Discussion

The present study evaluated the relationships among NT-proBNP, fPL, and NLR in cats regardless of their clinical diagnosis. Previous studies in human medicine have suggested potential interactions between cardiac and pancreatic diseases, whereas evidence in feline medicine remains limited (Bexelius et al., 2013; Chung & Lin, 2017).

NT-proBNP is a well-established marker of myocardial stress and cardiac dysfunction in cats (Borgeat et al., 2018; Luis Fuentes et al., 2020), while fPL is considered one of the most reliable biomarkers for the diagnosis of feline pancreatitis (Forman et al., 2021; Dulude et al., 2024). In the present study, no significant correlation was observed between NT-proBNP and fPL concentrations. These findings are consistent with those reported by Cridge et al. (2025), who similarly found no direct association between pancreatic lipase and NT-proBNP concentrations in cats. Although cardiopancreatic interactions have been described in human medicine, the current results suggest that elevations in these biomarkers may occur independently in feline patients.

The neutrophil-to-lymphocyte ratio has gained increasing attention as an indicator of systemic inflammation and disease severity in both cardiac and inflammatory disorders (Yurtdas et al., 2018; Fries et al., 2022). However, no significant correlations were identified between NLR and either NT-proBNP or fPL in the present study. This finding suggests that NLR alone may have limited utility for identifying concurrent cardiac and pancreatic involvement in cats.

Several limitations should be considered when interpreting these results. The relatively small sample size may have reduced the statistical power of the analyses. In addition, acute and chronic pancreatitis cases were not evaluated separately, and other cardiac biomarkers, such as cardiac troponin I, were not included. Future prospective studies involving larger populations and additional biomarkers are needed to further clarify the relationship between cardiac and pancreatic disease in cats.

No significant associations were identified among NT-proBNP, fPL, and NLR in the cats included in this study. Although NT-proBNP and fPL

remain valuable biomarkers for the assessment of cardiac and pancreatic disorders, respectively, their combined evaluation did not demonstrate a meaningful relationship under the conditions of the present study.

These findings suggest that the diagnosis of concurrent cardiac and pancreatic disease should not rely solely on biomarker assessment but should be supported by clinical examination, diagnostic imaging, and additional laboratory testing. Further large-scale prospective studies are warranted to better elucidate the interactions between cardiac dysfunction, pancreatic disease, and systemic inflammation in cats.

Conflict of Interest

The outhors declared that there is no conflict of interest.

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Research Article

An Investigation of Stress Factor That Pose a Risk for Lower Urinary Tract Diseases in Cats

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Abstract

This study was designed as a prospective, controlled, questionnaire-based case-control study to investigate stress-related risk factors for lower urinary tract diseases in cats. The study was conducted between 01.04.2026 and 01.09.2026 in veterinary clinics in Ankara, Turkey. Thirty-eight cats diagnosed with lower urinary tract disease were included as the patient group, and 31 healthy cats constituted the control group. The study was approved by the Kırıkkale University Local Ethics Committee for Animal Experiments (Decision No: 20, dated 25.03.2026). Data were collected using a 30-item questionnaire adapted from Cameron et al. (2004) and the MEMO protocol developed by Buffington et al. (2006), covering five main categories: demographic information, housing conditions, social environment, care practices, and owner profile. The questionnaire was administered through face-to-face interviews and via the Google Forms platform. No statistically significant differences were found between the patient and control groups regarding adoption age, current age, breed, sex and neuter status, body weight, number of cats in the household, feeding type, living area, daily routine regularity, and temperament characteristics. However, several noteworthy findings were obtained. A statistically significant difference was detected between groups in terms of litter box cleaning frequency ($p=0.022$; Cramer's $V=0.407$), with smart litter box use reported exclusively in the patient group. The presence of at least one clinical or behavioral problem in the past six months was significantly higher in the patient group ($p=0.045$; $OR=3.04$). Vocalization during urination ($p=0.019$) and hiding behavior ($p=0.034$) were reported at significantly higher rates in the patient group. Multiple correspondence analysis revealed that the two groups did not form completely distinct profiles; however, categories related to clinical symptom reporting and behavioral responses were positioned closer to the patient group.

These findings suggest that the evaluation of lower urinary tract diseases in cats should encompass not only clinical signs but also housing conditions, care practices, behavioral responses, and stress-related factors.

Keywords: Cat, feline lower urinary tract disease, FLUTD, feline idiopathic cystitis, stress factors, questionnaire study, risk factors

Introduction

Feline lower urinary tract diseases (FLUTD) refer to a group of multi-etiological conditions in cats that affect the lower urinary tract and present with variable clinical symptoms such as dysuria, hematuria, pollakiuria, stranguria, periuria (inappropriate urination), anorexia, and/or urethral obstruction (Gerber et al., 2005; Buffington et al., 2006; Forrester & Roudebush, 2007).

FLUTD is not a diagnosis; it is a clinical syndrome associated with multiple etiological causes. For this reason, proper management of the differential diagnosis process is of critical importance. The terms FUS (Feline Urological Syndrome) and feline lower urinary tract disease were introduced in the 1970s and 1980s to describe cats suffering from urinary tract disorders. Unfortunately, in veterinary medicine, these terms have come to imply a specific disease for some practitioners, whereas these conditions actually encompass the possibility of multiple different diagnoses. Over the years, new diagnoses and terms have been added to this syndrome; in the 1990s, new etiologies such as feline idiopathic cystitis and interstitial cystitis were identified and included. (Elliott et al., 2017).

The most common cause of lower urinary tract disease in cats is idiopathic cystitis. When healthy cats are exposed to stressful events, they initially release catecholamines but return to normal after the adaptation process. In cats with FIC, however, they exhibit a prolonged catecholamine response that lasts longer than normal, similar to anxiety disorders in humans. (Shaer, 2018). Studies conducted over the past twenty years have shown that FIC can develop rapidly when a susceptible individual/cat is exposed to a "stimulating environment." It has been determined that susceptible individuals possess more vulnerability factors (genetic, environmental) compared to resilience factors in response to environmental stimuli. This situation may increase the sensitivity of the central stress response system (SRS) to threats. The term "stimulating environment" refers to a setting where the animal's perception of threat exceeds its sense of control (i.e., where it experiences "stress"), and this can lead to chronic, sustained activation and heightened sensitivity of the SRS. (Elliott et al., 2017).

It is anticipated that the findings will lay the groundwork for developing evidence-based preventive recommendations in veterinary practice in Turkey and contribute to the creation of owner education programs. The study's contribution to science can be evaluated from several perspectives. First and foremost, it will generate a unique dataset by revealing the relationship between Turkey-specific dietary habits, housing conditions, and owner profiles and FLUTD. Furthermore, the systematic measurement of stress factors using a standardized method based on the questionnaire developed by Cameron and colleagues (2004) represents a methodological innovation; the findings obtained have the potential to contribute to the development of practical preventive recommendations for clinicians and to strengthen an evidence-based approach in veterinary practice in Turkey. This study also qualifies as one of the first prospective controlled studies in Turkey to address stress factors as the primary variable.

Material and Method

This study was designed as a prospective, controlled field study conducted between April 1, 2026, and September 1, 2026, at veterinary clinics in the province of Ankara that hold valid operating permits. A total of 69 cats were included in the study, and the animals were divided into two groups: the patient group (n=38) and the control group (n=31). The patient group consisted of cats diagnosed with lower urinary tract disease, while the control group consisted of healthy cats with clinical examination and laboratory findings within normal limits.

The patient group consisted of cats diagnosed with FLUTD by experienced veterinarians at the veterinary clinics where they were treated, based on clinical findings. The diagnosis was based on a detailed medical history and physical examination findings—dysuria, hematuria, pollakiuria, stranguria, or urethral obstruction—and all FLUTD subtypes, including feline idiopathic cystitis, urolithiasis, and urethral obstruction, were included in the study. The owner's consent to participate in the study was also among the inclusion criteria. Cases with concurrent systemic disease, signs of active infectious disease, a history of antibiotic or immunosuppressive medication use within 30 days prior to examination, cats younger than 6 months of age, and cases where the owner did not complete the questionnaire in full were excluded from the study.

All cats included in the study underwent clinical

examination and diagnostic evaluation according to a standard protocol. All animals were subjected to a systematic physical examination by experienced veterinarians; hydration status, bladder palpation findings, and a general systemic assessment were recorded.

Urine samples were collected preferably via cystocentesis; when this was not possible, they were obtained via free-flow or catheterization. Urine samples underwent strip analysis and microscopic sediment examination. In strip analysis, pH, specific gravity, protein, glucose, ketones, bilirubin, nitrite, leukocytes, and erythrocytes were evaluated; in microscopic examination, the presence of erythrocytes, leukocytes, epithelial cells, crystals, bacteria, and casts was investigated. Urine culture and antibiotic susceptibility testing were performed in cases deemed necessary.

Physicians performing the examination conducted B-mode abdominal ultrasonography on the group of cats deemed necessary. The Sonoscape E2V Digital Color Doppler Ultrasound System (Sonoscape Medical Corp., Shenzhen, China) was used for ultrasonographic evaluation. B-mode abdominal ultrasonography was performed on all cases; bladder wall thickness, intraluminal echogenicity, the presence of sand and stones within the lumen, and the urethral region along with other abdominal organs were systematically examined.

Results

When examining the ages at which cats were adopted, the highest rate in the patient group was observed among cats adopted between 2 and 5 months of age. 31.6% of the cats in the patient group were adopted during this period. In the control group, the adoption rate for the 2–5-month age group was similarly high, at 32.3%. In the control group, the adoption rate for cats adopted between 5 and 8 weeks of age appears to be higher than in the patient group. This category accounted for 35.5% in the control group, compared to 23.7% in the patient group. In contrast, the adoption rate for cats in the 5–12-month and 1-year-and-older age groups was higher in the patient group compared to the control group. Specifically, the adoption rate for cats 1 year and older was 18.4% in the patient group and 6.5% in the control group. However, no statistically significant difference was found between the patient and control groups regarding the age at adoption ($\chi^2 = 4.90$; df = 4; p = 0.298). Nevertheless, cats adopted at a later age were relatively more represented in the patient group.

In both groups, it was observed that cats were most frequently adopted from the street. The proportion of cats adopted from the street was 39.5% in the patient group and 38.7% in the control group. The rate of adoption from friends or acquaintances was higher in the patient group (28.9%) than in the control group (16.1%). In contrast, adoption via social media appears to be higher in the control group (19.4%) than in the patient group (5.3%). Adoption through pet shops is low in both groups. Overall, no statistically significant difference was found between the patient and control groups regarding the source of cat adoption (χ^2=4.193; df=4; p=0.380). This finding indicates that, in the current sample, the source of adoption is not a distinct variable that alone explains the difference between the patient and control groups.

When the overall distribution is evaluated, the proportion of middle-aged and older cats in the patient group appears to be relatively higher than in the control group. Specifically, cats aged 9 years and older accounted for 26.3% of the patient group and 19.4% of the control group. However, no statistically significant difference was found between the patient and control groups in terms of the current age distribution. This result indicates that, in the current sample, age is not a distinct factor that alone explains the distinction between the patient and control groups.

No statistically significant difference was found between the patient and control groups in terms of breed distribution (χ^2=5.134; df=7; p=0.644). The most common breed in both groups was the Turkish Angora. Turkish Angora cats accounted for 63.2% of the patient group and 67.7% of the control group. This finding indicates that Turkish Angora cats are predominant in the study population and are represented in similar proportions in the patient and control groups. The British Shorthair breed was present at a rate of 15.8% in the patient group and 16.1% in the control group, showing a very similar distribution between the two groups. The Scottish Fold breed accounted for 13.2% of the patient group and 9.7% of the control group. Other breeds were represented at low frequencies and were observed in limited numbers within the total sample.

Table 1. Relationship with the outdoor

Frequency of visitors	Patient n (%)	Control n (%)	Total n (%)
Occasionally	15 (39.5)	17 (54.8)	32 (46.4)
Rarely	19 (50.0)	8 (25.8)	27 (39.1)
Frequently	3 (7.9)	6 (19.4)	9 (13.0)
Constantly	1 (2.6)	0 (0.0)	1 (1.4)
Total	**38 (100.0)**	**31 (100.0)**	**69 (100.0)**

Table 2. Routine Acitvation

Daily routine order	Patient n (%)	Control n (%)	Total n (%)
Yes, quite regular	19 (50.0)	15 (48.4)	34 (49.3)
No, irregular	1 (2.6)	2 (6.5)	3 (4.3)
Partially regular	18 (47.4)	14 (45.2)	32 (46.4)
Total	**38 (100.0)**	**31 (100.0)**	**69 (100.0)**

Discussion

In this study, cats diagnosed with lower urinary tract disease were compared with a healthy control group in terms of demographic characteristics, care habits, environmental factors, and behavioral traits. The findings revealed that while the patient and control groups largely exhibited similar profiles, significant differences were observed between the groups in variables such as litter box cleaning frequency, history of problems in the past six months, meowing while urinating, and hiding behavior. In this section, the findings were discussed within the framework of relevant literature; the study's strengths and weaknesses, as well as its clinical significance, were evaluated.

No statistically significant difference was found between the groups in terms of current weight distribution (Figure 3.7 and Table 3.7). The predominance of the 3–5 kg weight range in the patient group (50.0%) and the higher representation of cats weighing over 7 kg in the patient group compared to the control group (7.9% vs. 3.2%) align with the obesity-FLUTD association (Figure 3.7 and Table 3.7). The literature reports a significant increase in the risk of FLUTD in obese cats; this is emphasized as being associated with a sedentary lifestyle, reduced frequency of bladder emptying, and chronic inflammation originating from adipose tissue (Cameron et al., 2004; He et al., 2022). However, in the current study, the fact that weight information is based on owner reports and that weight was asked in a categorical manner limits the sensitivity of this variable. These results are consistent with the literature.

Based on the data from this study, it was observed that the vast majority of cats in both groups did not live with another animal species, and no significant difference was found (Figure 3.9 and Table 3.9). The rate of cohabitation with dogs was found to be higher in the control group compared to the patient group. This finding suggests that the presence of another animal species does not constitute a significant risk for FLUTD. However, it should not be forgotten that individual cat personality and adaptability may play a decisive role in this relationship (Buffington, 2011).

This study compared cats diagnosed with lower urinary tract disease with a healthy control group in terms of demographic characteristics, care practices, environmental factors, clinical signs, and behavioral characteristics. The findings can be summarized as follows:

However, notable differences were observed between the groups in certain variables. A statistically significant difference was found between the groups regarding the frequency of litter box cleaning; in particular, the fact that the use of smart litter boxes was reported only in the patient group and the differences in the distribution of cleaning frequency categories were noteworthy. The prevalence of experiencing at least one clinical or behavioral problem in the past six months was found to be significantly higher in the patient group compared to the control group; the likelihood of being in the patient group was approximately three times higher among cats reporting problems. When symptoms reported in the past six months were evaluated individually, meowing while urinating and hiding behavior were found to be

statistically significantly higher in the patient group. Symptoms such as changes in urine color and behavioral changes were observed at a higher rate in the patient group but did not reach the threshold for statistical significance.

Given the sample size and methodological limitations of this study, the findings should be interpreted not as causal relationships but as potential clinical and behavioral trends. To more comprehensively establish the relationship between FLUTD and stress factors in the feline population in Turkey, it is recommended that prospective studies be conducted with larger sample sizes, involving multiple centers, and based on standardized diagnostic protocols.

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Research Article

Asssessment of Whole Blood Parameters in Healthy Cats Under Enviromental Cigarette Smoke Exposure

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Abstract

The study aimed to evaluate whole blood parameters in healthy cats exposed to environmental tobacco smoke. Because humans and pets share living spaces, the potential effects of tobacco smoke on pets are becoming increasingly important. The study compared healthy domestic cats exposed to smoke (ETS) with those not exposed (NE). It is known that tobacco smoke has toxic, mutagenic, and carcinogenic effects on living organisms through various exposure routes classified as first-, second-, and third-hand smoke. According to the study's findings, the mean corpuscular hemoglobin (MCH) level in the ETS group was found to be statistically significantly higher than that in the control group ($p=0.017$). Additionally, red blood cell distribution width (RDW) and mean platelet volume (MPV) were found to be significantly lower in the ETS group than in the control group ($p = 0.039$ and $p = 0.041$, respectively). No statistically significant differences were observed between the groups regarding other complete blood count parameters (RBC, HGB, HCT, MCV, MCHC, WBC, LEN, MON, GRAN, PLT, PDW, PCT). In conclusion, this study demonstrated that environmental cigarette smoke exposure in healthy cats can lead to limited but statistically significant changes in complete blood count parameters. In particular, the differences in MCH, RDW and MPV values highlight the need for further research to investigate cats' hematological responses to environmental cigarette smoke. These findings highlight the potential risks of environmental tobacco smoke exposure to pet health and underscore the need for more sensitive biomarkers and long-term follow-up studies..

Keywords: Environmental cigarette smoke, cat, whole blood, passive smoking, Hematology

Introduction

Humans and companion animals increasingly share the same living environments, resulting in exposure to common environmental health risks. Among these, tobacco smoke remains one of the most important and preventable causes of disease worldwide. Tobacco smoke exposure has been associated with the development of numerous pathological conditions, disease progression, delayed recovery, reduced treatment efficacy, and increased mortality (Max et al., 2012; Lindsay et al., 2016; Onor et al., 2017).

There is no safe level of tobacco smoke exposure. Its harmful effects extend beyond carcinogenesis and involve multiple organ systems, including the respiratory, cardiovascular, nervous, endocrine, and immune systems (Lindsay et al., 2016; Tan et al., 2021). Tobacco smoke exposure is commonly classified into three categories: first-hand smoke (direct exposure of the smoker), second-hand smoke (sidestream smoke inhaled by nearby individuals),

and third-hand smoke, which results from residual tobacco-related contaminants persisting on surfaces and in the environment after smoking has ceased (R. Baker & Proctor, 1990; Woodward & Al-Delaimy, 1999). Exposure to second-hand and third-hand smoke is collectively referred to as environmental tobacco smoke.

Companion animals living in smoking households are continuously exposed to enviromental tobacco smoke and may therefore experience adverse health effects similar to those reported in humans. While the consequences of passive smoking have been extensively investigated in human populations, relatively few studies have focused on companion animals despite their prolonged and close contact with smokers (Puzycki et al., 2018). Nevertheless, experimental and veterinary studies suggest that tobacco smoke exposure may adversely affect animal health (Hutchinson, 2017).

One of the most consistently reported effects of tobacco smoke exposure in humans is the alteration of hematological parameters. Both active and passive smoking have been associated with increased total leukocyte (white blood cell; WBC) counts, reflecting tobacco smoke-induced systemic inflammation (Ronchetti et al., 1990; Bridges et al., 1993; Panagiotakos et al., 2004). However, whether similar hematological changes occur in companion animals remains poorly understood.

Given the widespread prevalence of tobacco use and the lifelong exposure of many pets to environmental tobacco smoke, further investigation of its effects on companion animals is warranted. Therefore, the aim of the present study was to evaluate the impact of chronic tobacco smoke exposure on hematological parameters in clinically healthy domestic cats living in smoking households. By identifying potential hematological alterations associated with tobacco smoke exposure, this study seeks to contribute to the growing body of evidence regarding the health consequences of environmental tobacco smoke in veterinary medicine.

Material and Method

Study Cohort

A total of 40 clinically healthy domestic cats without any apparent clinical signs or health complaints were included in the study. According to owner-reported anamnesis, 20 cats that were not exposed to tobacco smoke and 20 cats that were regularly exposed to tobacco smoke were selected. These animals comprised the study population.

Clinical Examination Protocol

The study population consisted of cats presented to the Internal Medicine Clinic of the Animal Hospital, Faculty of Veterinary Medicine, Kırıkkale University, for routine health examinations and vaccination purposes. Prior to vaccination or the performance of any diagnostic procedures, all animals underwent a comprehensive clinical examination.

During the clinical evaluation, body temperature, respiratory rate, and heart rate were recorded. Subsequently, peripheral lymph nodes were systematically examined, and capillary refill time was assessed in each animal. Temperament and responsiveness to environmental stimuli were also evaluated. Dermatological examination was performed to assess skin integrity and to determine the presence or absence of pruritus. Thoracic auscultation was conducted to evaluate cardiac and pulmonary sounds, and particular attention was given to the detection of any pathological auscultatory findings. In addition, mucosal membranes were examined, and normal mucosal coloration was confirmed.

Only cats with clinical parameters within established reference ranges were included in the study. Additional inclusion criteria comprised the absence of lymphadenopathy, gastrointestinal signs such as diarrhea and vomiting, respiratory signs including coughing, exercise intolerance, and any abnormal cardiac or pulmonary sounds detected during auscultation. Thus, only clinically healthy cats with no evidence of systemic disease were enrolled in the study.

Cats Not Exposed Cigarette Smoke (NE)

Information regarding tobacco smoke exposure was obtained through owner-reported anamnesis. Owners were asked whether they or any other household members smoked within shared living spaces or in areas directly accessible to the cat. Additionally, information was collected concerning the cat's lifestyle, including whether it visited other households, was allowed outdoors, or was temporarily cared for by other individuals. In cases where the cat was entrusted to another caregiver, the smoking status of those individuals was also recorded.

Based on these assessments, cats were assigned to the non-exposed group only if no history of tobacco smoke exposure could be identified across all evaluated environments. Accordingly, cats with no direct or indirect contact with tobacco smoke in their primary residence or in any other regularly visited setting were included in the non-exposed (control) group.

Cats Exposed Cigarette Smoke (ETS)

Cats presented for routine vaccination and general health examinations were classified as tobacco smoke-exposed based on information obtained from owner-reported anamnesis. Animals were included in the exposed group when the owner or other household members regularly smoked within shared living

environments. Exposure was considered present regardless of whether smoking occurred directly in the cat's immediate vicinity, provided that tobacco products were consumed within areas shared by the cat. Furthermore, cats that were temporarily housed in other residences, entrusted to caregivers, or had regular contact with environments where smoking occurred were also classified as exposed.

Accordingly, cats with a history of potential exposure to environmental tobacco smoke through either second-hand smoke or third-hand smoke were assigned to the tobacco smoke-exposed group. This classification was based on the presence of tobacco smoke exposure in any environment frequented by the animal, including the primary household and other regularly visited locations.

Collecting Samples and Laboratory Analyses

For hematological assessment, whole blood samples were collected from cats in both the exposed and control groups via venipuncture of the cephalic vein (vena cephalica antebrachii). Blood was drawn aseptically according to standard procedures and placed into EDTA-containing tubes (2 ml) to prevent coagulation prior to laboratory analysis.

Whole blood samples collected in EDTA-anticoagulated tubes were subjected to hematological analysis. Complete blood count parameters were determined within 30 minutes of sample collection using an automated hematology analyzer (Mindray BC-5000, China) in accordance with the manufacturer's recommendations.

Statistical Analyses

Statistical analyses were performed using Jamovi software (version 2.6; Australia). The threshold for statistical significance was set at $p<0.05$. Data distribution was assessed using the Shapiro-Wilk test to evaluate normality. Variables demonstrating a normal distribution were expressed as mean ± standard deviation (SD), whereas non-normally distributed variables were presented as median and interquartile range (IQR). Comparisons between the tobacco smoke-exposed and control groups were conducted using the Student's t-test for parametrically distributed data and the Mann-Whitney U test for non-parametric data.

Results

The study evaluated the complete blood count results obtained from the SE and SDE groups. When the WBC, LEN, MON, and GRAN values were examined, no statistically significant differences were found between the SE and SDE groups. It was observed that the mean values for all parameters were similar across the groups (Table 1).

Table 1. Results of Leukogram

	SE		SDE		
	Mean (Min-Max)	**Median (IQR)**	**Mean (Min-Max)**	**Median (IQR)**	P Value
WBC	10.5 (3.00-18.00)	10.2 (7.78)	10.3 (4.10-17.50)	9.70 (5.22)	0.797
LEN	3.52 (0.90-9.30)	3.17 (3.55)	2.73 (0.50-7.50)	1.80 (3.55)	0.144
MON	0.35 (0.08-1.30)	0.25 (0.39)	0.39 (0.10-2.10)	0.25 (0.22)	0.662
GRAN	6.66 (1.30-14.0)	6.45 (6.04)	7.19 (2.30-15.10)	6.55 (7.38)	0.695

The data on RBC, HGB, HCT, MCV, MCH, MCHC, and RDW values for the SE and SDE groups were analyzed. No statistically significant differences were detected between the groups in RBC, HGB, HCT, and MCV values. In the SDE group, the mean values of RBC, HGB, HCT, and MCV were higher than those in the SE group, but no statistically significant difference was observed. Similarly, it was found that the MCHC value had a higher mean in the SDE group but did not show a statistically significant difference. For the MCH value, the mean in the SDE group was higher and statistically significant, while for the RDW value, the mean was lower than that of the SE group and statistically significant ($p<0.05$). Results was shown on table 2

Table 2. Results of Eritrogram

	SE		SDE		
	Mean (Min-Max)	**Median (IQR)**	**Mean (Min-Max)**	**Median (IQR)**	**P Value**
RBC	9.30 (6.23-12.10)	9.29 (2.17)	9.54 (5.96-13.30)	9.34 (2.08)	0.718
HGB	13.8 (9.10-18.2)	14.1 (3.03)	15.20 (10.4-19.7)	15.4 (4.30)	0.093
HCT	44.1 (27.60-64.10)	45.2 (14.4)	46.0 (20.4-66.4)	45.2 (16.2)	0.626
MCV	47.1 (38.9-57.6)	46.9 (9.57)	48.2 (34.3-60.6)	46.9 (7.99)	0.626
MCH	14.8 (13.1-20.5)	14.7 (1.25)	16.0 (13.0-21.5)	16.1 (2.27)	**0.017**

MCHC	32.0 (27.1-51.0)	32.1 (6.00)	34.1 (26.7-53.8)	33.9 (8.05)	0.365
RDW	16.1 (12.9-20.4)	15.6 (3.05)	14.6 (12.7-16.7)	14.8 (1.92)	**0.039**

The study's findings regarding PLT, MPV, PDW, and PCT are summarized in table 3. It was observed that PLT and PDW values were higher in the SDE group compared to the SE group; however, no statistical difference was found between the groups. Regarding PCT values, it was determined that the mean value in the SDE group was lower than that in the SE group. However, no statistically significant difference was found between the groups for PCT values. For MPV values, it was determined that the mean value in the SDE group was lower than that in the SE group and exhibited a statistically significant difference.

Discussion

The present study investigated the potential effects of environmental tobacco smoke exposure on hematological parameters in clinically healthy domestic cats. Given the increasing coexistence of humans and companion animals within shared indoor environments, understanding the biological consequences of passive tobacco smoke exposure in pets has become an important veterinary and public health concern. Tobacco smoke contains numerous irritant, pro-inflammatory, mutagenic, and carcinogenic compounds that may affect both humans and animals through second-hand and third-hand exposure pathways (Baker et al., 2004; Matt et al., 2011; Ferrante et al., 2013).

No significant differences were observed between tobacco smoke-exposed and non-exposed cats with respect to leukogram parameters, including WBC, lymphocyte, monocyte, and granulocyte counts. Although active and passive smoking have been associated with leukocytosis in humans, all cats enrolled in the present study were clinically healthy and free of overt inflammatory or infectious conditions, which may explain the absence of detectable leukocytic changes (Ronchetti et al., 1990; Bridges et al., 1993; Panagiotakos et al., 2004). Furthermore, exposure classification was based on owner-reported anamnesis, preventing objective assessment of exposure intensity and duration. Therefore, the lack of significant leukogram alterations should not be interpreted as evidence of the absence of biological effects.

Among erythrogram parameters, RBC, HGB, HCT, MCV, and MCHC did not differ significantly between groups. However, these variables tended to be higher in tobacco smoke-exposed cats, and HGB values approached statistical significance. Carbon monoxide exposure is known to impair oxygen transport through carboxyhemoglobin formation and may stimulate compensatory erythropoietic responses during chronic exposure (Alkhedaide, 2020). Although these trends were not statistically significant, they may suggest subtle effects of environmental tobacco smoke on the feline erythroid system.

The most notable erythrogram findings were the significantly increased MCH and decreased RDW values observed in tobacco smoke-exposed cats. MCH reflects the average hemoglobin content of erythrocytes, whereas RDW represents variability in erythrocyte size (Fava et al., 2019). The biological significance of these findings remains uncertain; however, they suggest that environmental tobacco smoke exposure may be associated with alterations in erythrocyte indices even in clinically healthy animals. Given that both parameters may be influenced by age, nutritional status, physiological variation, and analytical factors, these findings should be interpreted cautiously and confirmed in larger studies.

Evaluation of platelet parameters revealed no significant differences in PLT, PDW, or PCT values. In contrast, MPV was significantly lower in the tobacco smoke-exposed group. MPV is considered an indicator of platelet size and production dynamics and has been associated with inflammatory and vascular processes in human smokers (Loffredo et al., 2025). However, because other platelet indices remained unchanged, the isolated reduction in MPV cannot be conclusively interpreted as evidence of platelet activation or clinically relevant hemostatic dysfunction. Nevertheless, this finding may indicate subtle alterations in platelet kinetics associated with environmental tobacco smoke exposure.

The present findings should be interpreted within the context of previous studies demonstrating that nicotine and cotinine concentrations in biological samples from cats and dogs correlate with household smoking exposure (Bertone et al., 2002; Groppetti et al., 2023). In addition, passive smoke exposure has been associated with several neoplastic diseases in companion animals, including lymphoma in cats and respiratory tumors in dogs (Reif et al., 1992; Pérez et al., 2014; Gervásio et al., 2021). Unlike those studies, the current investigation focused on clinically healthy cats and evaluated potential hematological alterations before the development of overt disease, thereby providing insight into possible early biological effects of environmental tobacco smoke exposure.

Several limitations should be acknowledged.

First, exposure assessment relied solely on owner-reported information and was not confirmed by biomarkers such as cotinine or nicotine. Second, the relatively small sample size limited statistical power and prevented subgroup analyses according to age, breed, and sex. Finally, the cross-sectional design precludes causal inference. Future studies should incorporate larger and more balanced populations, objective exposure biomarkers, and longitudinal follow-up to better characterize the hematological and clinical consequences of environmental tobacco smoke exposure in cats.

Overall, environmental tobacco smoke exposure was not associated with significant alterations in leukogram parameters; however, significant differences were detected in MCH, RDW, and MPV values. These findings suggest that chronic exposure to tobacco smoke may be associated with subtle hematological changes in otherwise healthy cats. From a clinical perspective, veterinarians should consider household smoking status during routine anamnesis and inform pet owners about the potential risks of second-hand and third-hand tobacco smoke exposure to companion animals.

Ethical Approval

This study was approved ethical status by the Kırıkkale University Local Ethics Committee on Animal Experiments (Decision Number: 2026/03/41)

Conflict of Interest

The outhors declared that there is no conflict of interest.

Authors' Contributions

This research was designed by MAY and YP. In Data collection adn processing part was made by MAY and YP. Analysis were made by YP. Literature review protocols were realised by MAY and YP. The article was written by MAY and YP.

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Case Report

A Case of *Staphylococcus pseudintermedius* in a Dog

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Abstract

This report presents a case of deep pyoderma in a 5-year-old dog presented with widespread skin lesions, anorexia, and poor general condition. Microbiological examination of skin scrapings isolated Staphylococcus pseudintermedius. The isolate was determined to be susceptible to amoxicillin-clavulanic acid, enrofloxacin, erythromicin, gentamicin, lincomycin, cefoperazone, rifamicin, cefoxitin, kanamycin, cefotaxime and trimethoprim-sulfamethoxazole, and resistant to oxytetracycline, vancomycin and doxycycline. Although initial treatment with amoxicillin-clavulanic acid based on the antibiogram showed temporary improvement, symptoms recurred; the subsequent combination of enrofloxacin and trimethoprim-sulfamethoxazole was ineffective. Due to the progressive deterioration of the patient's general condition, euthanasia was performed. The failure to achieve the expected clinical response despite using susceptible antibiotics was attributed to the chronicity of the disease, the widespread nature of the lesions, the patient's dehydration, and a weakened immune system limiting antibiotic efficacy.

Keywords: Dog, *Staphylococcus pseudintermedius*, Pyoderma, Antibiotic Resistance, Zoonosis.

Introduction

Staphylococci are frequently isolated in routine veterinary diagnostics (Sareyyüpoğlu et al., 2013). *Staphylococcus intermedius*, first described by Hajek (1976), has been defined as part of the normal skin and mucosal flora in various animals, including dogs, cats, pigeons, minks, horses, foxes, raccoons, goats, and gray squirrels (Vandenesch et al., 1995). Recent studies have shown that isolates phenotypically identified as *S. intermedius* actually belong to three different species: S. intermedius, *S. pseudintermedius* (Devriese et al., 2005), and *Staphylococcus delphini,* collectively referred to as the *Staphylococcus intermedius* group (SIG) (Bannoehr et al., 2007; Sasaki et al., 2007). According to this new classification, the species colonizing and causing infections in dogs and cats is not *S. intermedius*, but *S. pseudintermedius;* whereas *S. intermedius* primarily colonizes pigeons (Sasaki et al., 2007; Perreten et al., 2010).

S. pseudintermedius is a part of the commensal natural flora of the normal skin, hair follicles, and nasal, oral, and anal mucosae of healthy dogs (Rubin and Chirino-Trejo, 2011). However, it acts as an opportunistic pathogen in the presence of predisposing factors such as disruption of the skin

barrier, atopic dermatitis, surgical procedures, or immunosuppression. As a zoonotic and opportunistic bacterium, *S. pseudintermedius* is most commonly associated with animal diseases such as pyoderma, otitis externa, and keratoconjunctivitis (Tolar et al., 2006; Wang et al., 2012; Meroni et al., 2019; Roken et al., 2022). Pyodermas in dogs generally develop depending on local or systemic factors (Bubnov, 2025). Although the normal skin microbiota maintains tissue homeostasis, the disruption of the epithelial barrier for various reasons (allergic, hormonal, or traumatic) sets the stage for the overgrowth of opportunistic pathogens, allowing them to penetrate from the surface to deeper layers and cause purulent infections known as pyoderma. Localized crusted lesions have been observed in dogs, particularly in the inguinal and axillary regions (Summers et al., 2012; Yu and Vogelnest, 2012). Lesions usually begin on the fore and hind legs, groin, and axillary region. This situation may be due to these areas being more humid and providing a suitable environment for the proliferation of bacterial microorganisms (Grice and Segre, 2011).

Material and Method

The subject of this case report is a 5-year-old dog admitted to the Mehmet Akif Ersoy University Animal Hospital. In the case history, it was reported that the animal was kept in a garden and was presented to the hospital after widespread lesions developed on various parts of its body. Clinical examination revealed a deteriorated general condition, anorexia, secondary dehydration, and marked weakness due to inappetence (Figures 1 and 2).



Figure 1. Cutaneous lesions localized to the head region of the dog.



Figure 2. Generalized cutaneous lesions distributed throughout the body of the dog.

After evaluating the patient's general clinical status, a comprehensive laboratory examination was performed. Blood samples were collected under appropriate conditions for laboratory analysis. Serum biochemical analyses were performed using appropriate reagents with the DiaSys Respons 910 (Germany) autoanalyzer. Hematological analyses of blood samples collected in EDTA tubes were conducted using the Edan H60 Vet blood count device. For histopathological examination, skin tissue samples obtained from the lesions were fixed in 10% neutral buffered formalin for 3 days at room temperature. Following fixation, the tissues were processed through a graded ethanol series (70%, 80%, 90%, 96%, and 100%) for dehydration, cleared in xylene, and embedded in paraffin wax using a standard tissue processing system. Paraffin-embedded tissue blocks were sectioned at 5 µm thickness using a rotary microtome and mounted glass slides. The sections were then dried at 37–40°C overnight to ensure proper adhesion.

For hematoxylin–eosin staining, sections were first deparaffinized in xylene (2 × 5 min) and rehydrated through a descending alcohol series (100%, 96%, 80%, 70%) to distilled water. Slides were stained with Harris hematoxylin for 5–7 minutes, followed by rinsing in running tap water. Subsequently, sections were counterstained with eosin for 1–3 minutes, dehydrated through ascending alcohol series, cleared in xylene, and coverslipped using a permanent mounting medium.

Results

The analyses revealed an increase in leukocyte (WBC) and neutrophil (NEU) values (Table 1).

Table1. Results of hematological parameters.

Parameter	Result	Reference Range
WBC (10^9/L)	40.50	6.00 - 17.00
NEU (10^9/L)	38.16	3.62 - 12.30
NEU %	94.3	52.0 - 81.0
LYM (10^9/L)	1.20	0.83 - 4.91
LYM %	2.9	12.0 - 33.0
MON %	0.9	2.0 - 13.0
RBC (10^9/L)	6.46	5.10 - 8.50
HGB (g/L)	151	110 - 190
HCT %	47.5	33.0 - 56.0
PLT (10^9/L)	252	117 - 490

Serum levels of albumin (ALB), globulin, albumin/globulin ratio, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine (Crea), blood urea nitrogen (BUN), gamma-glutamyl transferase (GGT), total bilirubin, and total protein were measured (Table 2).

Table 2. Results of selected biochemical parameters.

Parameter	Result	Reference Range
ALB (g/dL)	2.05	2.90 - 3.80
ALP (U/L)	316	11 - 162
AST (U/L)	60	11 - 54
ALT (U/L)	32	24 - 97
BUN (mg/dL)	21	9 - 27
CREA (mg/dL)	0.70	0.40 - 1.80
GLUC (mg/dL)	65.1	61 - 115
TP (g/dL)	7.21	5.40 - 7.20

S. pseudintermedius was isolated from the microbiological culture performed on skin scrapings and hair samples. Antimicrobial susceptibility testing determined that the agent was resistant to oxytetracycline, doxycycline, and vancomycin. In contrast, it was found to be susceptible to the amoxicillin-clavulanic acid combination, erythromycin, gentamicin, enrofloxacin, lincomycin, cefoperazone, rifamycin, trimethoprim-sulfamethoxazole, cefoxitin, and kanamycin (Table 3).

Table 3. Antibiotic susceptibility and resistance profile of *S. pseudintermedius*.

Antibiotic	Susceptible	Resistant
Amoxycillin + Clavulanic Acid (AMC)	X	
Erythromycin (E)	X	
Gentamicin (G)	X	
Enrofloxacin (ENR)	X	
Lincomycin (L)	X	
Cefoperazone (CFP)	X	
Rifamycin (RF)	X	
Oxytetracycline (OTC)		X
Trimethoprim / Sulfamethoxazole (SXT)	X	
Doxycycline (DO)		X
Cefoxitin (FOX)	X	
Kanamycin (K)	X	
Cefotaxime	X	
Vancomycin		X

Histopathological examination of lesioned skin samples revealed characteristic features consistent with pyoderma. In superficial lesions, the epidermis showed varying degrees of acanthosis, spongiosis, and orthokeratotic to parakeratotic hyperkeratosis. The dermis exhibited moderate to marked inflammatory cell infiltration, predominantly composed of neutrophils, along with lymphocytes, plasma cells, and occasional macrophages. In some areas, a mixed inflammatory infiltrate with increased mononuclear cells was evident. Perivascular dermatitis was commonly noted, accompanied by vascular dilation and congestion. In some sections, dermal edema and extravasation of erythrocytes were present, reflecting ongoing inflammation and vascular damage (Fig.3).

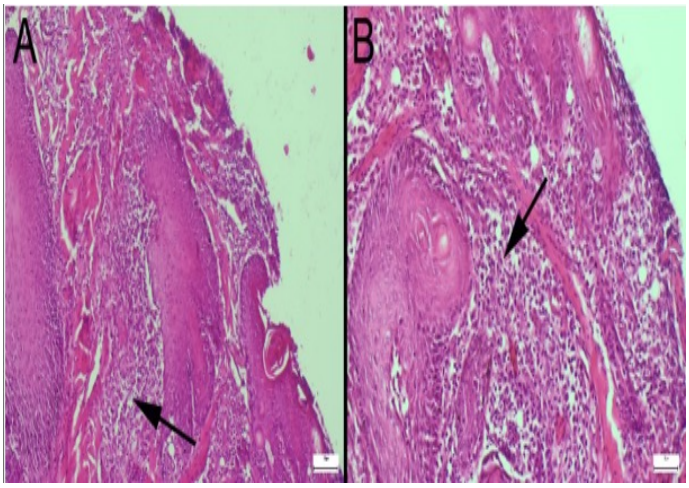


Figure 3. Histopathological appearance of the lesioned area. Marked inflammatory reaction in the dermal area (arrow), HE, scale bars= 100µm for A and 50µm for B.

Based on these findings, supportive therapy was initiated alongside an amoxicillin-clavulanic acid combination administered intramuscularly (IM) for 7 days, dosed according to the animal's body weight. During the first four days of treatment, a significant improvement was observed in the skin lesions and general clinical status; however, within the following three days, the lesions deteriorated again, and it was found that the appetite, which had initially improved, decreased once more.

For this reason, as a second-line treatment, a trimethoprim-sulfamethoxazole combination (in syrup form) was administered in the morning, and enrofloxacin was administered intramuscularly (IM) in the evening. However, no significant improvement in clinical findings was observed during this treatment process; on the contrary, a progressive worsening of the patient's general condition occurred. At this stage, euthanasia was performed in accordance with the owner's consent.

Discussion

S. pseudintermedius is a natural part of the skin and oral flora of healthy dogs (Wang et al., 2013). The pharynx, rectum, nose, and skin are among the most frequently colonized areas in dogs (Somayaji et al., 2016). Although humans are not natural hosts for this bacterium, it is reported that transient colonization can develop in individuals in contact with pets (Carroll et al., 2021).

In one study, it was reported that 46% of owners of dogs with deep pyoderma carried strains genetically identical to those in their dogs (Guardabassi et al., 2004). These data are consistent with literature findings indicating that transmission primarily occurs from dogs to humans, as the agent is a member of the natural flora of dog skin (Guardabassi et al., 2004). Transmission routes include direct physical contact as well as indirect exposure occurring through the home environment (Wang et al., 2013). Close contact situations, such as dogs licking their owners' faces or sharing the same bed, have been reported to increase the risk of infection in humans (Somayaji et al., 2016). While the agent frequently leads to bite wound infections in humans, it can also be the cause of serious invasive conditions such as surgical site infections, bacteremia, pneumonia, and brain abscesses (Wang et al., 2013; Somayaji et al., 2016). Particularly, comorbidities such as diabetes, peripheral vascular disease, and cardiovascular disease are among the important predisposing factors for these invasive infections (Somayaji et al., 2016; Ubiergo and Castro, 2021). Furthermore, the frequent confusion of this agent with *S. aureus* in microbiology laboratories frequently leads to the underdiagnosis (Sasaki et al., 2007; Perreten et al., 2010) or misidentification of cases in humans (Ubiergo and Castro, 2021).

The most significant public health concern regarding zoonotic transmission is the transfer of methicillin-resistant *S. pseudintermedius* (MRSP) strains (Tamakan and Göçmen, 2019). These resistant strains not only limit treatment options but also carry the risk of transferring resistance genes from canine isolates to human pathogen staphylococci through horizontal gene transfer (Guardabassi et al., 2004). Given the zoonotic potential of the agent and its capacity to transfer resistance genes, determining the antibiotic susceptibility profiles of strains isolated from dogs is of critical importance for both animal and public health.

The tetracycline group resistance detected in our study is consistent with many studies reported in the literature. Research has indicated that the agent is highly resistant to this antibiotic group, emphasizing that this resistance tends to increase over time (Hartmann et al., 2005; Morris et al., 2006; Lyskova et al., 2007; Vanni et al., 2009; Zamankhan Malayeri et al., 2010; Wang et al., 2012; Göçmen et al., 2020; Jung et al., 2020; Wang et al., 2022). Conversely, Müştak et al. (2020) detected tetracycline resistance in approximately half of the isolates, reporting a partially susceptible profile to this antibiotic group. In contrast, Keskin Yılmaz and Baş (2024) obtained results differing from our findings by reporting that canine isolates were susceptible to oxytetracycline.

Our findings regarding susceptibility to amoxicillin-clavulanic acid, enrofloxacin, and gentamicin parallel many international studies. It has been reported in research that these agents show high efficacy against canine isolates and that susceptibility rates are maintained at high levels (Vanni et al., 2009; Chrobak et al., 2011; Wang et al., 2012; Nocera et al., 2023). Indeed, Morris et al. (2006) supported our findings by reporting that susceptibility to gentamicin is highly preserved. Similarly, Chrobak et al. (2011) stated that all methicillin-susceptible strains were susceptible to amoxicillin-clavulanic acid and cephalothin, while resistance to gentamicin and ciprofloxacin remained at very low levels. The high gentamicin susceptibility detected in our study also aligns with other research reporting that staphylococci isolated from dogs are completely susceptible to this antibiotic (Hartmann et al., 2005; Lyskova et al., 2007). However, in the case in question, gentamicin treatment was not preferred due to the presence of severe dehydration associated with anorexia and malnutrition at the time of the patient's admission.

However, Müştak et al. (2020) detected a certain level of resistance to the amoxicillin-clavulanic acid combination, while Keskin Yılmaz and Baş (2024) partially diverged from our findings by reporting widespread resistance, especially to the use of amoxicillin alone. Nevertheless, although the amoxicillin-clavulanic acid combination was found to be susceptible in the antibiogram test in our case, clinical symptoms were observed to intensify again after the fourth day of treatment.

Loeffler et al. (2007) and Morris et al. (2006) have drawn attention to the possibility of resistance developing against enrofloxacin in chronic or resistant cases. However, in our case, although the agent was found to be susceptible to enrofloxacin according to the antibiogram results, no significant clinical improvement could be achieved in the second-line treatment. This situation is thought to be due to the chronicity of the case.

The trimethoprim-sulfamethoxazole susceptibility detected in our study is consistent with studies that found this agent effective in the literature (Morris et al., 2006; Vanni et al., 2009; Wang et al., 2012; Saputra et al., 2017; Göçmen et al., 2020; Müştak et al., 2020). In this case, the use of a trimethoprim-sulfamethoxazole combination together with enrofloxacin was preferred for second-line treatment because an adequate clinical response could not be obtained from the first-choice amoxicillin-clavulanic acid combination and the possibility of resistance development was considered.

Similarly, the susceptibility detected in our study against rifamycin, cefoxitin, cefoperazone, kanamycin, and cefotaxime is consistent with studies reporting that the agent is highly susceptible to these agents, and our findings are supported by the literature (Morris et al., 2006; Chrobak et al., 2011; Wang et al., 2022; Nocera et al., 2023).

On the other hand, the high erythromycin and lincomycin (clindamycin) susceptibility we detected in our study contrasts with the high resistance rates reported in the literature for these drugs. This, along with a study conducted in Poland reporting significant resistance to clindamycin, supports the uniqueness of our findings (Morris et al., 2006; Hauschild and Wójcik, 2007; Wang et al., 2012; Saputra et al., 2017; Wang et al., 2022; Nocera et al., 2023).

Lastly, the vancomycin resistance detected in our study was recorded as rare and noteworthy data that showed inconsistency with research reporting full susceptibility or no resistance to this drug in canine isolates (Vanni et al., 2009; Chrobak et al., 2011; Saputra et al., 2017; Jung et al., 2020; Nocera et al., 2023). Additionally, Müştak et al. (2020) emphasized that all isolates were fully susceptible to amikacin and that this agent could be an alternative treatment option in resistant cases.

Conclusion

In conclusion, although susceptibility to the relevant antibiotics was detected in the antibiogram, the expected clinical response could not be achieved in our study. This situation is thought to be related to the fact that the disease had become chronic at the time of the case's admission to our clinic, the lesions were spread throughout the body, and the general condition was deteriorated. Furthermore, it was considered that dehydration due to the inability to feed and the weakening of the immune response may have limited the effectiveness of the antibiotics determined to be susceptible.

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Research Article

Investigation of the Effectiveness of Continuous Renal Replacement Therapy (Crrt) on Blood Urea Nitrogen and Serum Creatinine Values in Dogs and Cats with Renal Failure

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Abstract

This study aimed to evaluate the effectiveness of continuous renal replacement therapy (CRRT) on blood urea nitrogen (BUN) and serum creatinine (CREA) levels in cats and dogs with renal failure where conventional treatment methods were insufficient. The distribution characteristics of the data were examined using the Shapiro-Wilk test, and it was determined that they did not show a normal distribution. Therefore, non-parametric statistical methods were applied. Comparisons between treatment groups (CRRT and medical treatment) were performed using the Kruskal-Wallis test, and pairwise post hoc comparisons were carried out using the Dunn test and Bonferroni correction for parameters showing significant differences. Changes over time in animals receiving CRRT treatment were examined using the Friedman test, and median values and time-dependent change curves were evaluated. Furthermore, the biochemical recovery time was analyzed using a Kaplan-Meier-like method. In cats, CRRT treatment resulted in a significant decrease in BUN, creatinine, and inorganic phosphorus levels; changes were more limited in the medical treatment group. In dogs, a decrease in parameters was also observed after CRRT, but some changes were not statistically significant. Kaplan-Meier analysis showed that biochemical recovery in cats occurred in approximately six hours for BUN and IP, and approximately five hours for creatinine. In dogs, creatinine recovered rapidly (approximately one hour), while BUN and IP returned to normal within six and four hours, respectively. CRRT offers a significant therapeutic advantage over conventional medical treatment in cases of severe renal failure, particularly in cats, by enabling faster and more significant recovery of important renal biomarkers.

Keywords: Renal Failure, CRRT, Blood Urea Nitrogen, Cat, Dog, Serum Creatinine, Continuous Renal Replacement Therapy

Introduction

Chronic kidney disease (CKD) is a significant health problem commonly seen in both cats and dogs, resulting from progressive kidney disease that typically develops over months or even years (Şahin and Çamkerten, 2003). The disease is characterized by irreversible and progressive impairment of nephron function, regardless of the initial cause. Although CKD is most commonly associated with older animals, it can also be observed in animals of different age groups. In acquired cases, the disease may stem from pathological processes occurring in

various structures of the kidney. These processes may affect the glomeruli, renal tubules, interstitial tissues, or the vascular structures supplying the kidney. However, in many clinical cases, it may not be possible to definitively determine the underlying cause of the disease (Börkü and Kurtdede et al., 2000).

Various diseases that may contribute to the development of CKD in pets have been identified. These include glomerulonephritis, bacterial pyelonephritis, diabetes mellitus, polycystic kidney disease, chronic urinary tract obstructions, nephrolithiasis, kidney damage caused by various nephrotoxic substances, and certain immune-mediated kidney diseases (Çelik and Bal, 2002). In particular, renal lymphoma is also considered a significant aetiological factor in cats. The damage occurring in the nephrons is permanent, and the loss of function is irreversible. This situation, over time, leads to the involvement of healthy nephrons as well and to a progressive deterioration in kidney function. The loss of nephrons triggers fibrotic processes in the renal tissue, and the areas containing damaged nephrons are gradually replaced by connective tissue. In cases where a significant proportion of nephrons is lost, renal atrophy, capsular adhesions, and structural changes may be observed. Furthermore, various pathological changes may develop during this process, including inflammation, fibrosis, hypertrophy, mineralization, tubular atrophy, glomerular sclerosis, mononuclear cell infiltration, and degeneration of the tubular epithelium (Altıntaş et al., 2006).

In the later stages of the disease, it is not only the kidney tissue that may be affected, but other organ systems as well. In particular, the rise in blood urea levels associated with chronic kidney failure can lead to the development of degenerative changes in the gastrointestinal system. These pathological changes are most commonly observed in the stomach and intestinal tissues (Börkü et al., 2000).

The diagnosis of chronic kidney disease requires a combined assessment of clinical evaluations and laboratory findings. In addition to hematological and biochemical analyses, the diagnostic process also utilizes electrocardiographic, radiographic, ultrasonographic, and pathological examinations. Among the hematological findings, increases in white blood cell count, reticulocyte count, and mean corpuscular volume (MCV) are frequently observed, while decreases in red blood cell count and hematocrit are noteworthy. In serum biochemical analyses, significant increases in urea, creatinine, phosphorus, and potassium levels may be detected. Urinalysis may reveal findings such as decreased urine specific gravity, haematuria, and albuminuria. Sediment examinations may identify epithelial cells, crystals, various cellular structures, and filamentous formations. Furthermore, microbiological examination of urine samples has isolated various bacterial pathogens, including Staphylococcus spp., Streptococcus spp., Proteus mirabilis, Klebsiella pneumoniae, and Pseudomonas aeruginosa (Börkü et al., 2000).

The development of extracorporeal blood purification methods dates back to the early 20th century. Experiments conducted by a pharmacologist at the Johns Hopkins University School of Medicine laid the foundations for the dialysis techniques used today. In these experiments, arterial blood taken from animals was mixed with an anticoagulant, passed through a system consisting of semi-permeable membranes, and then returned to the circulation. This system comprised semi-permeable membranes formed by pipette-like tubes situated within a liquid medium. The researchers demonstrated that the chemical properties of the blood could be altered by changing the composition of the surrounding fluid. This method, defined as ‘live diffusion’ or ‘live dialysis’, is based on the principle of diffusion and forms the basis for subsequent intermittent hemodialysis (IHD) applications (Acierno, 2011).

Continuous renal replacement therapy (CRRT), on the other hand, is a more recently developed method of extracorporeal blood purification. As the name suggests, this method is administered continuously

and continues until kidney function improves or the patient switches to intermittent dialysis treatment. In CRRT, as in intermittent hemodialysis, the patient's blood is passed through a dialyzer composed of semi-permeable membrane fibers. However, unlike intermittent hemodialysis, in CRRT the removal of solutes is achieved not only by diffusion but also through convection and, to a more limited extent, adsorption mechanisms (Bayendur and Acar, 2024)

One of the key advantages of CRRT is that, thanks to the slow and continuous administration of treatment, electrolyte balance and acid-base balance can be controlled more stable. The principle of continuous operation ensures that the treatment has an effect closer to that of physiological kidney function. Furthermore, thanks to the convection mechanism, it is possible to remove larger molecules from the blood that are difficult to eliminate by diffusion alone. These molecules are closer in size to the substances filtered by healthy kidneys. In contrast, intermittent hemodialysis aims to rapidly alter uremic toxins, fluid balance, and acid-base status within a short period. Consequently, the procedure requires the production of large volumes of pure dialysate, which necessitates complex infrastructure equipped with specialised water purification systems (Özkalıpcı, 2025).

In CRRT, however, the combined diffusion and convection mechanisms allow the use of pre-prepared sterile replacement fluids. This reduces the need for complex water purification systems and significantly simplifies maintenance requirements between treatments (Acierno, 2011).

Cases of renal failure, which are frequently encountered at veterinary clinics, animal hospitals and practices in Turkey, generally require complex management and long-term treatment protocols, regardless of whether they present as acute or chronic. During this process, elevated blood urea nitrogen and serum creatinine levels lead to various clinical symptoms due to difficulty controlling them; this results in progressive deterioration of the patient's general condition and, in advanced stages, irreversible tissue damage. Disruption of osmotic balance due to nephron loss may lead to various cardiovascular complications (Börkü et al., 2000).

Imbalances in electrolyte levels lead to systemic effects. The aim is to reduce blood urea nitrogen (BUN) and creatinine levels—which are used for diagnosis and monitoring in renal failure—as quickly as possible without causing permanent loss of nephrons in the renal tissue (Şentürk et al., 2003).

In this study, however, the aim was to comparatively evaluate the changes in high BUN and creatinine levels—which cannot be sufficiently reduced by methods such as parenteral fluid therapy—following continuous renal replacement therapy (Kang et al., 2024).

Material and Method

Animal Material

This study was conducted in accordance with Decision No. 32 of 15 November 2023 issued by the Local Ethics Committee for Animal Experiments at Kırıkkale University.

The patient material for this study consisted of a minimum of 10 cats and 10 dogs brought to the Research and Application Hospital of the Faculty of Veterinary Medicine at Kırıkkale University; these were animals in which a diagnosis of renal failure had been established following an assessment of biochemical parameters in relation to their symptoms.

Following investigations that included a complete blood count, blood biochemical parameters, and blood gas measurements, radiographic and ultrasonographic examinations were performed; diagnoses were made based on these findings, and patients were identified for whom CRRT was indicated**.**

Laboratory Tests

Diagnosis of Renal Failure

Patients included in the study, regardless of age, were assessed based on clinical examination findings, particularly those with a history of loss of appetite, fatigue, vomiting, bad breath, reduced urine output, and sudden deterioration in general condition. Patients presenting with these symptoms were screened using a CBC (complete blood count) to assess for suspected conditions such as vomiting, reduced urine output, dehydration, hypovolaemia–hypervolemia, and hypothermia–hyperthermia; at this stage, HCT, WBC, RBC, HGB, and MCHC values were considered. In patients suspected of having renal failure, biochemical parameters such as BUN, CREA, IP, and Na-K-Cl were first examined from the samples taken to assess renal function. Based on these parameters, radiography and ultrasonography were performed for differential diagnosis.

Prior to continuous renal replacement therapy, the cats' CBC, biochemical parameters and blood gas measurements were recorded. During treatment, BUN, CREA, IP, K and blood gas measurements were taken at the start, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 10 hours and 12 hours, depending on the progress of the treatment and the patient's condition**.**

Hematological Analyses

To perform hematological analyses on the cats and dogs in the study group, 1 ml of blood was collected into EDTA-containing tubes and 3 ml into anticoagulant-free tubes from the cephalic vein of the forearm. To obtain serum samples, the blood collected in anticoagulant-free tubes was centrifuged immediately at 3000 rpm for 10 minutes. The separated serum samples were transferred to Eppendorf tubes and stored at -20°C. Blood samples collected in EDTA tubes were analyzed using a hematology analyzer (Mindray®, VH5R) to perform a complete blood count. The results were recorded.

Blood samples collected in tubes without anticoagulant (FUJI DRI-CHEM NX500IC) were analyzed using a biochemistry analyzer to measure biochemical parameters. The results were recorded. Blood gas measurements were performed using a blood sample syringe containing dry heparin (EDAN I15VET device). The results were recorded.

Continuous Renal Replacement Therapy

Prior to the procedure, an intravenous catheter was inserted into the cephalic vein of the forearm. This was followed by intravenous sedation. An intratracheal tube was inserted to prepare for gas anaesthesia. Isoflurane was selected as the anaesthetic gas.

Prior to the procedure, bidirectional catheterisation of the jugular vein was performed in cats and dogs. The BAXTER PRISMAFLEX device was used for this procedure.

Statistical Analyses

SPSS 10.0 was used to analyze numerical data for variables identified in cats and dogs. The arithmetic mean (X) and standard deviation (SX) of the parameters were calculated. Differences at a significance level of $P \leq 0.05$ were considered statistically significant.

The distribution characteristics of the data obtained in the study were assessed using the Shapiro–Wilk normality test. The analysis revealed that the data did not follow a normal distribution; consequently, non-parametric statistical methods were employed. The Kruskal–Wallis test was applied to compare biochemical parameters between the different treatment groups (CRRT, medical treatment and healthy control). Following the Kruskal–Wallis analysis, which detected statistically significant differences among the parameters, the Dunn multiple comparison test was used to perform pairwise comparisons between groups; a Bonferroni correction was applied to reduce the likelihood of Type I errors from multiple comparisons. The Friedman test, a non-parametric method suitable for repeated measurements, was used to evaluate changes in biochemical parameters over time in animals receiving CRRT. To assess treatment efficacy, the difference between the baseline value before treatment and the post-treatment value was calculated for each individual, and this change was expressed as the Δ (delta) value. The Δ value was calculated using the following formula:

$$\Delta = \text{Value_final} - \text{Value_initial}$$

A Kaplan-Meier-like analytical approach was used to assess the duration of biochemical recovery following CRRT treatment. In this analysis, recovery was defined as a reduction of at least 50% in the relevant biochemical parameter compared with its baseline value. Using this approach, the time interval during which the effect of CRRT treatment on biochemical parameters began to emerge was assessed. All data obtained in the study are presented as the median (IQR: interquartile range). Statistical analyses were performed using R Studio (Version 4.3.3), and a p-value of <0.05 was considered statistically significant.

Results

The distribution of pre-treatment biochemical parameters in the cats and dogs included in the study was assessed, and the initial values between the groups were examined (Table 1).

Table 1. Distribution of Initial Biochemical Parameters

Species	Parameter	Group	Median	Q1	Q3
Cat	BUN	CRRT	140	130	140
		Medical	57	36	72
	CRE	CRRT	10,21	7,00	12,65
		Medical	2,52	1,96	4,63
	IP	CRRT	11,60	10,20	15,00
		Medical	5,00	4,30	7,10
Dog	BUN	CRRT	122	103	140
	CRE	CRRT	9,40	8,03	10,58
	IP	CRRT	11,20	10,05	14,50

The study assessed whether there was a statistically significant difference in the distribution of biochemical parameters between different treatment groups in cats and dogs (Table 2).

Table 2. Results of the Kruskal-Wallis Test

Species	Parameter	H	p
Cat	BUN	10.65	0.001
Cat	CRE	10.40	0.001
Cat	IP	6.81	0.009
Dog	BUN	0.56	0.450
Dog	CRE	2.53	0.110
Dog	IP	1.72	0.180

Statistically significant differences were found between the groups in cats with regard to BUN, creatinine (CRE) and inorganic phosphorus (IP) levels ($p<0.05$). In contrast, no statistically significant differences were found between the groups in dogs for the biochemical parameters assessed ($p>0.05$). These results demonstrate that renal function indicators, particularly in cats, differ markedly between the treatment groups. The results of the multiple-comparison analyses, conducted to determine which treatment groups were responsible for the differences between groups, are presented in Table 3.

Table 3. Results of pairwise comparisons between groups regarding biochemical parameters in cats

Species	Parameter	Comparison	p*
Cat	BUN	CRRT vs Medical	0,004
Cat	CRE	CRRT vs Medical	0,004
Cat	IP	CRRT vs Medical	0,031

Statistically significant differences were found between the CRRT and medical treatment groups in cats in terms of BUN, creatinine (CRE) and inorganic phosphorus (IP) levels ($p<0.05$). The results obtained indicate that the levels of these biochemical parameters differ between cases receiving CRRT and those receiving medical treatment.

The changes in biochemical parameters over time in cats and dogs receiving CRRT are shown in Table 4, whilst the time-dependent change curves for these biochemical parameters are shown in Figure 1.

Table 4. Results on the Time-Dependent Changes in Biochemical Parameters During CRRT Treatment

Species	Parameter	χ^2	p
Cat	BUN	28,63	<0,001
	CRE	29,66	<0,001
	IP	24,00	<0,001
Dog	BUN	10,17	0,11
	CRE	11,03	0,08
	IP	11,95	0,06

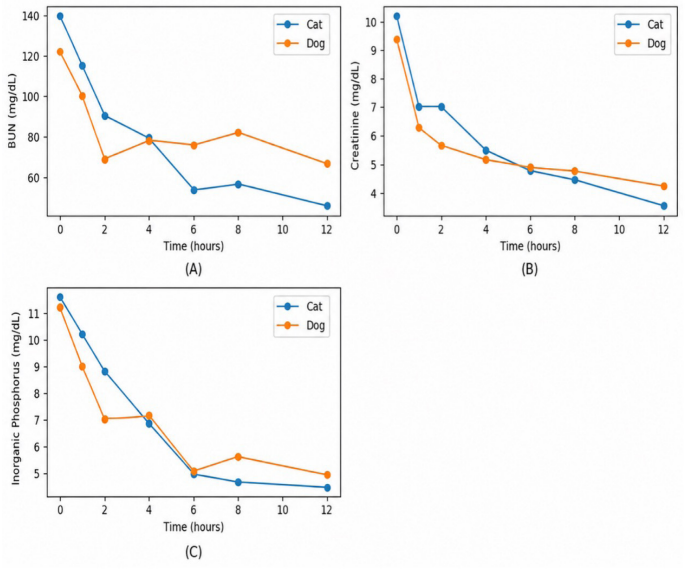


Figure 1. Time-dependent changes in biochemical parameters during CRRT treatment.
(A) The time-dependent changes in BUN levels, (B) the time-dependent changes in creatinine levels and (C) the time-dependent changes in inorganic phosphorus levels are shown for cats and dogs.

It was found that BUN, creatinine (CRE) and inorganic phosphorus (IP) levels in cats undergoing CRRT showed statistically significant changes over time ($p<0.001$). In dogs, however, the changes observed over time in BUN, creatinine and phosphorus levels were not statistically significant ($p > 0.05$). Nevertheless, it is noteworthy that p-values for some parameters in dogs were close to the threshold for statistical significance. These findings indicate that biochemical changes associated with CRRT treatment are more pronounced in cats in particular.

The changes (Δ) in biochemical parameters between pre-treatment and post-treatment levels for the treatments examined in the study were evaluated, and the results are presented in Table 5 and Figures 2 and 3.

Table 5. Changes Before and After Treatment (Median)

Species	Parameter	CRRT Δ	Medical Δ	p	Effect size (r)
Cat	BUN	-76	+15	0.004	0.72
	CRE	-5.4	+0.7	0.004	0.70
	IP	-7.2	-0.1	0.031	0.48
Dog	BUN	-48	—	0.450	0.12
	CRE	-3.1	—	0.110	0.19
	IP	-3.9	—	0.180	0.15

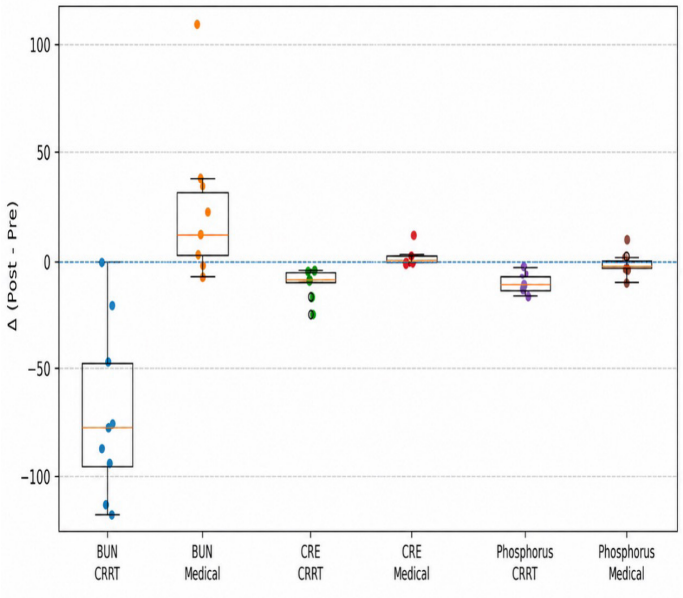


Figure 2. Changes in Biochemical Parameters in Cats Undergoing CRRT and Medical Treatment

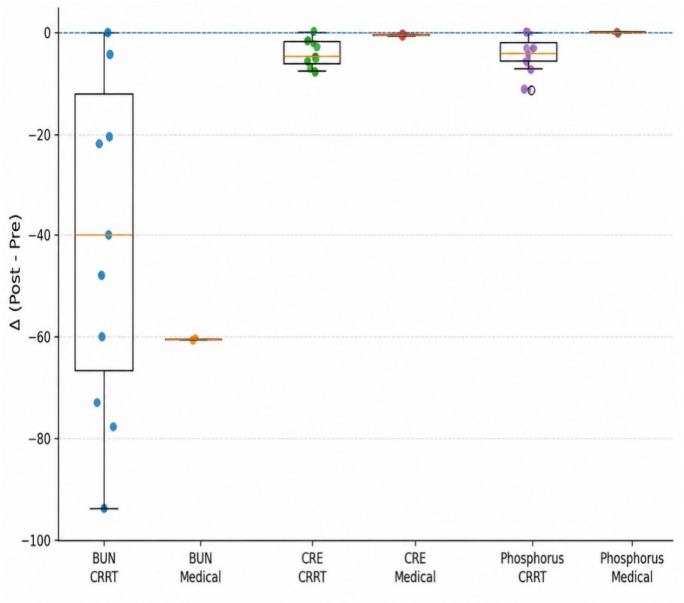


Figure 3. Changes in Biochemical Parameters in Dogs Undergoing CRRT and Medical Treatment

A marked reduction in BUN, creatinine (CRE), and inorganic phosphorus (IP) levels was observed in the group of cats receiving CRRT. Whilst a median reduction of 76 mg/dL in BUN levels was observed in cats receiving CRRT, an increase in this parameter was noted in the medical treatment group. Similarly, a median decrease of 5.4 mg/dL in creatinine levels was observed in the CRRT group, whilst a slight increase was noted in the medical treatment group. A median decrease of 7.2 mg/dL in inorganic phosphorus levels was also observed in the CRRT group. The results of the statistical analysis indicate that these differences are significant. It was determined that the differences between the CRRT and medical treatment groups in the BUN and creatinine parameters were statistically significant ($p=0.004$ and $p=0.004$, respectively) and had a high effect size (Cohen's $r=0.72$ and Cohen's $r=0.70$). A statistically significant difference was also observed between the groups in inorganic phosphorus levels ($p = 0.031$), with a moderate effect size (Cohen's $r = 0.48$).

A downward trend in biochemical parameters was also observed in dogs following CRRT treatment. In dogs, a median decrease of 48 mg/dL in BUN levels, a reduction of 3.1 mg/dL in creatinine levels and a decrease of 3.9 mg/dL in inorganic phosphorus levels were observed. However, it was found that these changes observed in dogs were not statistically significant ($p > 0.05$). When effect sizes were examined, it was seen that the effect on biochemical parameters in dogs was of a low magnitude (Cohen's $r = 0.12–0.19$).

When these findings are considered together, it is

evident that CRRT treatment is effective in reducing biochemical parameters in cases of renal failure; however, this effect is particularly more prominent and pronounced in cats.

The duration of the improvement in biochemical parameters observed in animals undergoing CRRT treatment was assessed, and the results are presented in Table 6.

Table 6. Median Duration of Biochemical Recovery Following CRRT Treatment

Species	BUN	CRE	IP
Cat	6 hours	5 hours	6 hours
Dog	6 hours	1 hour	4 hours

In cats undergoing CRRT, the median time for a 50% reduction in biochemical parameters compared with baseline values was found to be approximately 6 hours for BUN and IP, and approximately 5 hours for creatinine. In dogs, however, an earlier improvement in creatinine levels was observed, with the median time to improvement determined to be 1 hour. For BUN and IP parameters, the median times to improvement were calculated as 6 hours and 4 hours, respectively. These findings demonstrate that CRRT treatment has a marked effect on biochemical parameters within a short period of time.

To evaluate the temporal aspect of the effect of CRRT treatment on biochemical parameters, biochemical recovery times were analysed using the Kaplan-Meier approach. In this analysis, recovery was defined as a reduction of at least 50 per cent in the relevant biochemical parameter compared to its baseline value. The median recovery times and confidence intervals obtained from the analysis are presented in Table 7.

Table 7. Time to Biochemical Recovery Following CRRT Treatment (Kaplan-Meier Analysis)

Species	Parameter	Recovery time (hours)	%95 CI	Recovered animal (n)
Cat	BUN	6	4-8	9
	CRE	5	4-6	9
	IP	6	4-8	9
Dog	BUN	6	4-8	6
	CRE	1	1-2	6
	IP	4	3-6	6

According to the results of the Kaplan-Meier analysis, the median time to biochemical recovery for BUN and IP parameters in cats receiving CRRT was 6 hours, whilst for creatinine it was 5 hours. An examination of the Kaplan-Meier curves reveals that improvement in the biochemical parameters became particularly evident within the first 6 hours of treatment (Figure 4).

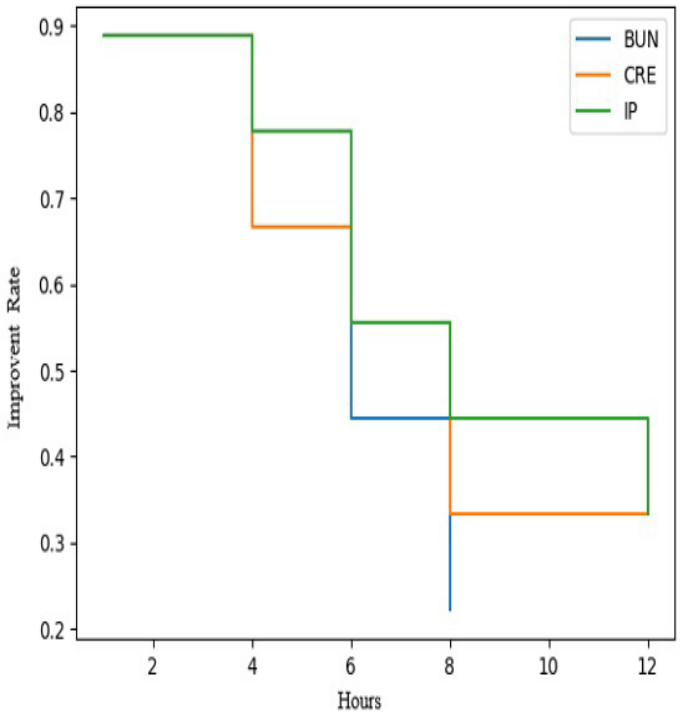


Figure 4. Changes in Biochemical Parameters Over Time in Cats During CRRT Treatment

On examination of the figure, a marked trend towards improvement in biochemical parameters over time is observed in cats receiving CRRT treatment. In particular, a downward trend in BUN, creatinine (CRE) and inorganic phosphorus (IP) levels becomes apparent from the very first hours of treatment. When the recovery curves shown in the graph are evaluated, a faster trend of improvement is observed, particularly in IP levels, during the early stages of treatment. The rapid decline observed in the IP parameter during the first few hours suggests that inorganic phosphorus responds more quickly to CRRT treatment than the other biochemical parameters. As for creatinine levels, improvement becomes apparent in the middle hours. In contrast, the decline in BUN levels is more gradual, and improvement becomes apparent in the later hours. Overall, it is evident that CRRT treatment has a rapid effect on biochemical parameters in cats and leads to marked biochemical improvement, particularly within the first 6–8 hours. This finding demonstrates that CRRT is an effective method for removing metabolic waste products in cats with acute kidney injury.

According to the Kaplan-Meier analysis, the median time to biochemical recovery for the creatinine (CRE) parameter was 1 hour in dogs receiving CRRT. In contrast, the median time to recovery was calculated as 6 hours for the BUN parameter and 4 hours for the inorganic phosphorus (IP) parameter. An examination of the Kaplan-Meier curves reveals that biochemical improvement in creatinine levels occurred more rapidly in the early stages of treatment, whilst improvement in the BUN and IP parameters followed a more gradual course (Figure 5).

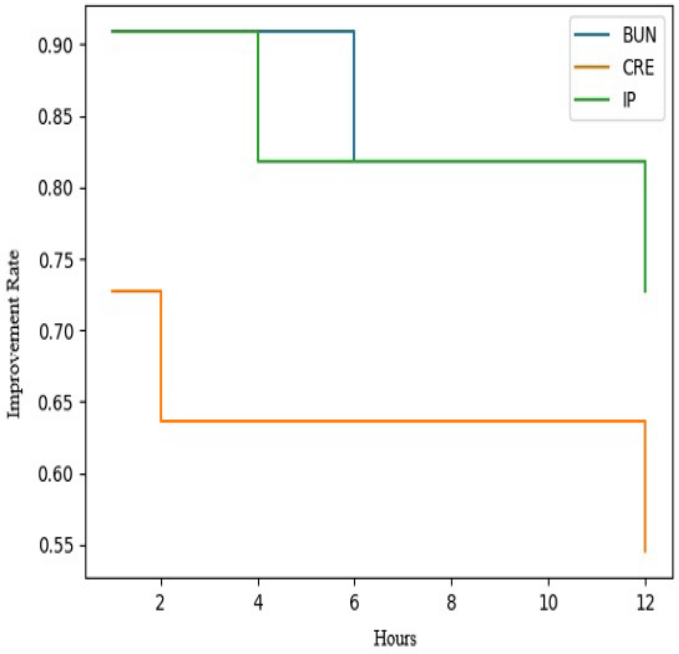


Figure 5. Changes in Biochemical Parameters Over Time in Dogs During CRRT Treatment

It has been observed that biochemical parameters in dogs tend to return to normal levels over time following CRRT treatment. However, it is noteworthy that this recovery follows a more gradual course compared to the changes observed in cats. An analysis of the curves in the graph reveals that inorganic phosphorus (IP) levels remain relatively stable during the early stages of treatment, and the rate of recovery is largely maintained. As for BUN levels, a gradual change in the rate of recovery is observed after the first few hours. In terms of creatinine (CRE) values, it is noteworthy that the change was more limited throughout the treatment period, and the rate of improvement remained lower than for the other parameters. Overall, it appears that CRRT treatment has a positive effect on biochemical parameters in dogs; however, this effect manifests more slowly and to a lesser extent than in cats. This suggests that CRRT treatment may elicit different biochemical responses between species.

When the findings of the study were evaluated overall, it was determined that there were significant differences between the CRRT and medical treatment groups in terms of baseline biochemical parameters. It was observed that BUN, creatinine and inorganic phosphorus levels were higher in cats receiving CRRT compared with the medical treatment group. In contrast, no statistically significant differences were detected between the groups in dogs with respect to baseline biochemical parameters. When changes in biochemical parameters over time were examined in animals receiving CRRT, BUN, creatinine, and inorganic phosphorus levels decreased significantly in cats during treatment. In dogs, whilst a time-dependent downward trend was observed in the same parameters, these changes were not statistically significant. An analysis of the changes between pre- and post-treatment values showed that a marked decrease in BUN, creatinine, and inorganic phosphorus levels occurred in the group of cats receiving CRRT. In the group receiving medical treatment, however, more limited changes in biochemical parameters were observed. In dogs, a downward trend in biochemical parameters was also observed following CRRT treatment. An evaluation using the Kaplan-Meier method revealed that biochemical recovery occurred within approximately 6 hours for BUN and inorganic phosphorus (IP) parameters, and within approximately 5 hours for creatinine in cats. In dogs, however, an earlier biochemical improvement in creatinine levels was observed, with a median time to improvement of approximately 1 hour. For the BUN and IP parameters, the times to recovery were approximately 6 and 4 hours, respectively.

Discussion

This study aimed to evaluate the effects of continuous renal replacement therapy (CRRT) on BUN, creatinine, and inorganic phosphorus levels in cats and dogs with chronic kidney failure. The findings obtained in this context are as follows:

An assessment of pre-treatment biochemical parameters in the cats and dogs included in the study revealed significant differences in baseline levels between the CRRT and medical treatment groups. In the group of cats receiving CRRT, the median BUN was 140 mg/dL (Q1: 130, Q3: 140), the median creatinine (CRE) was 10.21 mg/dL (Q1: 7.00, Q3: 12.65) and the median inorganic phosphorus (IP) level was 11.60 mg/dL (Q1: 10.20, Q3: 15.00). In the medical treatment group, the median BUN was 57 mg/dL (Q1: 36, Q3: 72), the median creatinine was 2.52 mg/dL (Q1: 1.96, Q3: 4.63) and IP 5.00 mg/dL (Q1: 4.30, Q3: 7.10). These results suggest that the cats in the CRRT group had a more advanced degree of renal dysfunction compared with those receiving

medical treatment. In dogs, however, only the baseline biochemical values of cases included in the CRRT group were assessed, and the median BUN was 122 mg/dL (Q1: 103, Q3: 140), creatinine 9.40 mg/dL (Q1: 8.03, Q3: 10.58) and IP 11.20 mg/dL (Q1: 10.05, Q3: 14.50). These values demonstrate that elevated biochemical parameters were also observed in dogs prior to treatment. These findings are consistent with the mean urea levels of 450 ± 25 mg/dL and creatinine levels of 5.9 ± 2 mg/dL reported by Börkü et al. (2000) in cats, and the mean urea levels of 370 ± 12 mg/dL and creatinine levels of 6.2 ± 3 mg/dL reported in dogs. It has been stated that, in chronic kidney disease, nitrogenous metabolic waste products such as urea and creatinine accumulate in the blood due to the progressive decline in kidney function, leading to the development of azotaemia and uraemia (Grollman & Grollman, 1959; Squires, 1996). It has been reported that these biochemical changes are closely related to the clinical severity of the disease and may be accompanied by systemic symptoms such as vomiting, loss of appetite, weight loss and lethargy (English, 1973; Squires, 1996). In clinico-pathological studies conducted on cats, findings such as azotaemia, hyperphosphataemia, metabolic acidosis and non-regenerative anaemia have been commonly observed, and it has been emphasised that these parameters are important indicators in the assessment of the disease (DiBartola et al., 1987). In our study, the high baseline BUN, creatinine and inorganic phosphorus levels observed in cats undergoing CRRT support the conclusion that these cases had a more advanced degree of renal dysfunction. Previous studies have found azotemia to be common in cats with chronic kidney disease, with a high prevalence reported, particularly in native short-haired cats of stray origin (King et al., 2007). These findings are consistent with the elevated BUN and creatinine levels in animals with azotemia reported by Geist et al. (2011), Elliott (2010), Jepson et al. (2009), and Chew et al. (2005). Furthermore, elevated serum phosphorus levels have been identified as an early indicator of azotemia by Jepson et al. (2009) and Squires (2007), whilst Little et al. (2007) reported that hyperphosphataemia is frequently observed in cats with urinary tract obstruction. In this context, the high baseline values observed in cats and dogs in the CRRT group are consistent with the literature and reflect the degree of renal dysfunction prior to treatment.

These findings are consistent with the results from CRRT in six dogs, as reported by Han et al. (2015). In that study, high BUN levels prior to the start of treatment (in some cases >150 mg/dL) were gradually reduced with CRRT, and a marked decrease in pre-BUN values was observed over the first five sessions. Treatment efficacy, as assessed by the urea reduction ratio (URR), was reflected in good clinical outcomes, particularly in dogs with an URR range of 57–72%; however, one case with severe azotaemia and electrolyte imbalance was lost after the third session. This finding demonstrates that an early response to treatment is important in predicting clinical outcomes and that CRRT safely supports renal function through gradual urea clearance. These high baseline values indicate the presence of marked azotaemia prior to CRRT and are consistent with the median BUN and creatinine values reported by Chen, Klainbart, Kelmer and Segev (2023) in dogs with acute kidney injury prior to CRRT. Chen et al. (2023) reported that CRRT is a safe and effective treatment, with urea levels decreasing gradually over time and renal function improving in a controlled manner. The study also emphasized that treatment efficacy can be optimized and complications minimized through appropriate dose management and control of the hourly urea reduction rate (URR). Furthermore, the complications reported in that study (filter occlusion, electrolyte and acid-base imbalances) were consistent with the limited hemodynamic and anticoagulation-related complications observed in our study. In this context, CRRT contributes to the rapid and safe improvement of biochemical parameters in cases of severe renal failure in both cats and dogs.

The study investigated the distribution of biochemical parameters in cats and dogs across different treatment groups. In cats, statistically significant differences were observed between the groups in terms of BUN ($H=10.65$; $p=0.001$), creatinine (CRE) ($H=10.40$; $p=0.001$) and inorganic phosphorus (IP) ($H=6.81$; $p=0.009$) levels; this finding is consistent with studies in the literature reporting increased BUN and creatinine levels in cats with azotaemia (Geist et al., 2011; Elliott, 2010; Jepson et al., 2009; Chew et al., 2005). Similarly, it has been reported that serum phosphorus levels rise in cats developing azotaemia and that hyperphosphataemia is frequently observed, particularly in cases of postrenal azotaemia (Jepson, 2009; Squires, 2007; Little, 2007). In dogs, however, no significant differences were observed between groups in BUN ($H=0.56$; $p=0.450$), creatinine ($H=2.53$; $p=0.110$) and IP ($H=1.72$; $p=0.180$) levels; this finding supports studies suggesting that baseline biochemical values in dogs may be more variable depending on the severity of azotaemia (Malouin et al., 2007; Drobatz et al., 2008). The increase in BUN and creatinine, hyperphosphataemia and changes in

biochemical parameters in cats with azotaemia are consistent with previous studies indicating that these vary depending on the type of azotaemia (Squires, 2007; Little, 2007; Jepson, 2009; Geist et al., 2011; Elliott, 2010; Chew et al., 2005). Furthermore, proteinuria is associated with azotaemia, and the urine protein-to-creatinine ratio has been reported as an important parameter in the assessment of biochemical changes (Lees, 2005; Grauer, 2011). The absence of intergroup differences in dogs suggests that the effect of kidney disease on biochemical parameters may be less pronounced compared to cats, or that baseline values may have limited statistical power (Malouin et al., 2007; Drobatz et al., 2008). These findings support the report by Börkü et al. (2000) that elevated urea and creatinine levels are associated with clinical signs (vomiting, dehydration, weakness, changes in urination).

In chronic renal failure, a reduction in glomerular filtration rate leads to the accumulation of nitrogenous metabolic waste products such as urea and creatinine in the blood, resulting in azotaemia and the development of the uraemic syndrome; these biochemical changes are associated with the clinical severity of the disease, and in advanced cases, metabolic disorders and systemic clinical symptoms may arise (English, 1973; Grollman & Grollman, 1959).

In our study, the elevated BUN, creatinine and IP values observed across the treatment groups in cats suggest that, in some cases, renal function loss is at an advanced stage.

A multiple comparison analysis of biochemical parameters between the CRRT and medical treatment groups in cats revealed statistically significant differences in BUN, creatinine (CRE) and inorganic phosphorus (IP) levels (BUN: $p=0.004$; CRE: $p=0.004$; IP: $p=0.031$). These results indicate that, in cats undergoing CRRT, baseline BUN, creatinine and IP values were higher compared to those in cats receiving medical treatment. In dogs, however, no pairwise comparisons between groups could be performed, and no statistically significant differences were found in terms of baseline parameters ($p>0.05$); this finding supports the notion that baseline biochemical values in dogs may be more variable depending on the severity of azotaemia, and that no marked differences may arise between groups prior to treatment (Malouin et al., 2007; Drobatz et al., 2008). The literature reports that conditions such as heart failure, acute and chronic renal failure, urinary tract obstructions, diabetes mellitus and dehydration in cats lead to increased BUN and creatinine levels (Malouin et al., 2007; Syme et al., 2006; Drobatz et al., 2008). Similarly, BUN and creatinine levels are elevated in cats with kidney disease and are associated with clinical signs (King et al., 2007; Boyd et al., 2008). It has been noted in the literature that phosphorus levels rise in cases of postrenal azotaemia and urinary tract obstruction (Jepson et al., 2009; Elliott, 2010; Little, 2007), and the high IP values observed in the CRRT group in our study are consistent with these findings. Furthermore, it has been reported that anemia is common in animals with chronic kidney disease (CKD) and that this develops as a result of reduced erythropoietin production (Cowgill, 1992). Uremia can lead to complications such as inflammation, edema, and ulceration of the gastrointestinal tract; this condition may manifest as clinical signs such as anorexia, vomiting, and diarrhea (Krawiec, 1996). It has also been emphasized that CKD is prevalent in older cats and dogs, and that abnormalities in biochemical parameters reflect the severity of the disease (Rubin, 1997). Clinical studies have reported that findings such as vomiting, anemia, a dull coat, and changes in urine are associated with elevated biochemical parameters; these findings support the contribution of CRRT treatment to biochemical and clinical improvement (Börkü et al., 2000).

When the time-dependent changes in biochemical parameters were evaluated in cats and dogs undergoing CRRT treatment, statistically significant decreases were observed in cats for BUN ($\chi^2=28.63$; $p<0.001$), creatinine (CRE) ($\chi^2=29.66$; $p<0.001$), and inorganic phosphorus (IP) ($\chi^2=24.00$; $p<0.001$) were observed in cats throughout the treatment period. These findings indicate that CRRT treatment leads to a marked improvement in biochemical parameters in cats and may contribute to clinical recovery. In dogs, however, changes in BUN ($\chi^2=10.17$; $p=0.11$), creatinine ($\chi^2=11.03$; $p=0.08$) and IP ($\chi^2=11.95$; $p=0.06$) were not found to be statistically significant; however, the fact that the p-values for some parameters were close to the threshold of significance suggests that CRRT may also have a gradual effect in dogs. Various studies in the literature have reported elevated levels of BUN, creatinine and phosphorus in cats with chronic and postrenal azotaemia (Geist et al., 2011; Elliott, 2010; Jepson et al., 2009; Chew et al., 2005; Malouin et al., 2007; Syme et al., 2006; Drobatz et al., 2008; King et al., 2007; Boyd et al., 2008; Little, 2007), and the elevated biochemical values observed in our study prior to CRRT are consistent with these findings. Furthermore, the literature reports that hyperkalaemia and electrolyte imbalances are common in cats with azotaemia and that, in some cases, these lead to ECG changes (Squires, 2007; Malouin et al., 2007; Greco

et al., 2007; Chew, 2011); in our study, hyperkalemia was also detected in cats with postrenal azotaemia, and certain electrocardiographic changes were observed. Changes in urine specific gravity and proteinuria in cats with azotaemia have been documented in the literature, and these parameters vary depending on the type of azotaemia (Lees, 2005; Grauer, 2011; Jepson, 2009; Little, 2007); in our study, the decrease in urine specific gravity in cats diagnosed with chronic kidney disease and the markedly elevated urine protein-to-creatinine ratio in cats with postrenal azotaemia are consistent with the literature. The significant decreases in BUN, creatinine and phosphorus levels observed during the course of treatment following CRRT have not yet been sufficiently reported in the literature; this can be regarded as an original contribution of our study and demonstrates that CRRT treatment provides rapid and effective biochemical recovery in cats. The fact that the changes observed in dogs were not statistically significant has been interpreted as a finding that requires support from a larger number of cases and long-term follow-up.

When the time-dependent changes in biochemical parameters were evaluated in cats and dogs undergoing CRRT treatment, statistically significant decreases were observed in cats for BUN (χ^2=28.63; p<0.001), creatinine (CRE) (χ^2=29.66; p<0.001) and inorganic phosphorus (IP) (χ^2=24.00; p<0.001) were observed in cats throughout the treatment period. These findings indicate that CRRT treatment leads to a marked improvement in biochemical parameters in cats and may contribute to clinical recovery. In dogs, however, changes in BUN (χ^2=10.17; p=0.11), creatinine (χ^2=11.03; p=0.08) and IP (χ^2=11.95; p=0.06) were not found to be statistically significant; however, the fact that the p-values for some parameters were close to the threshold of significance suggests that CRRT may also have a gradual effect in dogs. Various studies in the literature have reported elevated levels of BUN, creatinine and phosphorus in cats with chronic and postrenal azotaemia (Geist et al., 2011; Elliott, 2010; Jepson et al., 2009; Chew et al., 2005; Malouin et al., 2007; Syme et al., 2006; Drobatz et al., 2008; King et al., 2007; Boyd et al., 2008; Little, 2007), and the elevated biochemical values observed in our study prior to CRRT are consistent with these findings. Furthermore, the literature reports that hyperkalaemia and electrolyte imbalances are common in cats with azotaemia and that, in some cases, these lead to ECG changes (Squires, 2007; Malouin et al., 2007; Greco et al., 2007; Chew, 2011); in our study, hyperkalaemia was also detected in cats with postrenal azotaemia, and certain electrocardiographic changes were observed. Changes in urine specific gravity and proteinuria in cats with azotaemia have been documented in the literature, and these parameters vary depending on the type of azotaemia (Lees, 2005; Grauer, 2011; Jepson, 2009; Little, 2007); in our study, the decrease in urine specific gravity in cats diagnosed with chronic kidney disease and the markedly elevated urine protein-to-creatinine ratio in cats with postrenal azotaemia are consistent with the literature. The significant decreases in BUN, creatinine, and phosphorus levels observed during treatment following CRRT have not yet been adequately reported in the literature; this constitutes an original contribution of our study and demonstrates that CRRT provides rapid and effective biochemical recovery in cats. The fact that the changes observed in dogs were not statistically significant has been interpreted as a finding that requires support from a larger number of cases and long-term follow-up.

The effect of CRRT treatment on biochemical parameters in cats and dogs was assessed based on pre- and post-treatment change (Δ) values. In the group of cats receiving CRRT, the median BUN value decreased by 76 mg/dL, whilst a 15 mg/dL increase was observed in the medical treatment group (p=0.004; Cohen's r=0.72). Creatinine (CRE) levels showed a median decrease of 5.4 mg/dL in the CRRT group, whilst a slight increase of 0.7 mg/dL was observed in the medical treatment group (p=0.004; Cohen's r=0.70). A median decrease of 7.2 mg/dL in inorganic phosphorus (IP) levels was also observed in the CRRT group, whilst there was virtually no change in the medical treatment group (-0.1 mg/dL) (p=0.031; Cohen's r=0.48). A downward trend in BUN, creatinine and IP levels following CRRT was observed in dogs; however, this was not found to be statistically significant, and the effect sizes were calculated as 0.12, 0.19 and 0.15, respectively (p>0.05). These results are consistent with studies by Geist et al. (2011), Elliott (2010), Jepson et al. (2009), Chew et al. (2005), Squires (2007), and Little et al. (2007), which reported elevated BUN, creatinine, and phosphorus levels in cats with chronic and postrenal azotemia. It has been reported that hyperphosphatemia may worsen renal function and that a decrease in glomerular filtration rate may trigger the uremic syndrome (Zager, 1982; English, 1973; Rubin, 1997). The beneficial effect of a mineral-restricted diet on biochemical parameters and the pathophysiology of anemia-related abnormalities has also been highlighted in the literature. (Brown, O'Brien and Osborne, 1991; Cowgill, 1992). It has been noted in the literature that hyperkalaemia and electrolyte imbalances are common in cats with azotaemia and

may lead to ECG changes in some cases; in our study, hyperkalaemia was detected in cats with post-renal azotaemia and electrocardiographic changes were observed (Squires, 2007; Malouin et al., 2007; Di Bartola et al., 2006; Greco et al., 2007; Chew, 2011). Furthermore, it has been reported that urine specific gravity and the protein-to-creatinine ratio may vary depending on the type of azotaemia (Lees, 2005; Grauer, 2011; Jepson, 2009; Little, 2007); the low urine specific gravity and high protein-to-creatinine ratio observed in our study are consistent with the literature. A median decrease of 7.2 mg/dL in phosphorus in cats following CRRT supports findings in the literature that elevated phosphorus levels may be reversible with treatment. In terms of the severity of electrolyte imbalances and azotaemia, changes in potassium and bicarbonate levels are also consistent with indicators of response to treatment (Langston, 2008; Drobatz et al., 2008; Elliott et al., 2003). In conclusion, the significant reductions in BUN, creatinine and phosphorus observed with CRRT in cats can be regarded as an indicator of a rapid and effective response to treatment; the downward trend observed in dogs, meanwhile, supports the potential benefit of CRRT and demonstrates that changes in biochemical parameters in CKD can reliably reflect treatment efficacy and clinical response.

In cats undergoing CRRT, the median times to a 50% reduction in biochemical parameters from baseline were approximately 6 hours for BUN and inorganic phosphorus (IP), and 5 hours for creatinine (CRE). These findings indicate that CRRT has a marked effect on biochemical parameters in cats over a short period. In dogs, the median recovery times were calculated as 1 hour for creatinine, 6 hours for BUN, and 4 hours for IP; this indicates that creatinine responds more rapidly to CRRT treatment, whilst recovery in BUN and IP parameters follows a more gradual course. The high baseline values and clinical symptoms reported by Börkü et al. (2000) suggest that clinical recovery is possible alongside a reduction in biochemical parameters following treatment. Similar observations have also been reported in the literature. In a retrospective study by DiBartola et al. (1987), it was reported that biochemical changes in cats with CKD—such as azotaemia, hyperphosphataemia, metabolic acidosis and non-regenerative anaemia—were evaluated in conjunction with clinical and pathological findings. English (1973) and Grollman & Grollman (1959), meanwhile, discussed in detail the decline in glomerular filtration rate, the development of the uraemic syndrome and the occurrence of electrolyte and metabolic imbalances in chronic kidney disease. These studies support the view that the reduction in biochemical parameters observed with CRRT is consistent with the literature and that the treatment response may be clinically significant.

The effect of CRRT treatment on biochemical parameters in cats and dogs was assessed over time using Kaplan-Meier analysis. In this analysis, recovery was defined as a reduction of at least 50% in the relevant parameter compared with its baseline value. According to the Kaplan-Meier results, the median time to improvement in BUN and inorganic phosphorus (IP) levels was 6 hours, and to improvement in creatinine (CRE) levels was 5 hours in cats receiving CRRT. Examination of the curves revealed that biochemical improvement was particularly evident within the first 6 hours of treatment; IP levels showed a rapid decline in the early phase, creatinine became more pronounced in the middle hours, whilst BUN followed a more gradual course. In dogs, the median recovery times were calculated as 1 hour for creatinine, 6 hours for BUN and 4 hours for IP. These findings indicate that creatinine responds more rapidly to treatment in dogs, whilst improvements in BUN and IP levels follow a slower and more gradual course. In general, it can be said that CRRT has a rapid effect on biochemical parameters in cats, whereas in dogs the effect is variable and limited, depending on the parameter. These findings support the view that changes in biochemical parameters in chronic kidney disease (CKD) serve as indicators of treatment response. Similar results have been reported in the literature, and the positive effect of a mineral-restricted diet on biochemical parameters has been highlighted by Brown, O'Brien and Osborne (1991). The pathophysiology of anemia-related biochemical and clinical impairments has been explained by Cowgill (1992), whilst the reduction in glomerular filtration rate and the development of the uraemic syndrome in chronic kidney disease have been discussed in detail by English (1973) and Rubin (1997). The effect of hyperphosphataemia on worsening renal function was demonstrated experimentally by Zager (1982), whilst English (1973) and Grollman & Grollman (1959) detailed the relationship between increases in BUN and creatinine and the loss of renal function, as well as the metabolic and systemic consequences of chronic kidney disease (CKD). This body of literature demonstrates that the effect of CRRT in rapidly reducing biochemical parameters in cats and the parameter-dependent gradual improvement observed in dogs is grounded in pathophysiological principles.

The effect of CRRT treatment on biochemical

parameters in dogs was assessed over time using Kaplan-Meier analysis, and recovery was defined as a reduction of at least 50% in the relevant parameter compared with its baseline value. According to the analysis results, the median time to recovery for the creatinine (CRE) parameter was determined to be 1 hour, and a rapid improvement was observed in the early stages of treatment. In contrast, the median times to recovery for the BUN and inorganic phosphorus (IP) parameters were found to be 6 and 4 hours, respectively, and recovery followed a more gradual course. In a study by Chen et al. (2023), it was also reported that CRRT is a safe and effective treatment method in dogs with acute kidney injury, and that the response to treatment can be monitored using the hourly urea removal rate (URR) and biochemical parameters. The literature indicates that CRRT maintains haemodynamic stability and reduces the risk of dialysis disequilibrium syndrome (DDS) by removing solutes slowly and in a controlled manner (Cowgill, 2011; Cerdá & Ronco, 2009; Ronco et al., 1999; Davenport et al., 1993; Ronco, 2006; Gibney et al., 2008). CRRT efficacy is measured using URR and Kt/V values based on urea kinetic modelling (Basile et al., 2010; Ding et al., 2021; Chamney et al., 2014; Sridharan et al., 2017; Vanholder et al., 2019; Claure-Del Granado et al., 2012; Clark et al., 2017; Schiffl, 2020), with URR reflecting the rate of urea decline during treatment, whilst Kt/V is a more reliable indicator of overall treatment efficacy. Due to variability in urea production rate and solute clearance, URR alone may be insufficient in the later hours; therefore, its use in conjunction with Kt/V is recommended. In the literature, the complication profile of CRRT has been defined as circuit clotting, electrolyte and acid-base disturbances, and complications arising from heparin or citrate (Kovvuru & Velez, 2021; Tolwani, 2012; Brophy et al., 2005; McBride et al., 2019; Uchino et al., 2007; Akhoundi et al., 2015; Buduk et al., 2019; Coady et al., 2019). In our study, life-threatening complications were rare; hemodynamic instability or situations requiring intervention were observed in only a limited number of cases, which demonstrates that CRRT is a safe treatment method when properly monitored. In terms of clinical outcomes, the discharge rate following CRRT in dogs was 53%, with the majority of those discharged being followed up as non-dialysis-dependent (Segev et al., 2008; Segev et al., 2016). Similarly, the CRRT protocol based on the KDIGO guidelines, as reported by Raskansky et al. (2025), was found to be safe and effective in dogs, and it was emphasized that treatment response can be monitored using biochemical parameters. The international consensus guidelines published by Chen et al. (2026) also demonstrate that CRRT results in early and rapid reductions in CRE, alongside gradual decreases in parameters such as BUN and phosphorus, contributing both to the maintenance of haemodynamic stability and to the reduction of the risk of treatment-related complications. In this context, the changes in parameters observed in our study are consistent with the effects of CRRT described in the literature and support the view that the treatment is both safe and effective.

Conclusion

This study investigated the effects of continuous renal replacement therapy (CRRT) on high blood urea nitrogen (BUN) and creatinine (CREA) levels when traditional methods, such as parenteral fluid therapy, were insufficient. The data distribution was assessed using the Shapiro-Wilk test and found not to be normally distributed; therefore, non-parametric statistical methods were employed. Comparisons between treatment groups were performed using the Kruskal-Wallis test; for parameters with a statistically significant difference, post hoc pairwise comparisons were conducted using the Dunn test, and a Bonferroni correction was applied to reduce the risk of Type I error. Baseline biochemical measurements revealed significant differences between cats treated with CRRT and those receiving medical treatment alone. In particular, BUN, creatinine and inorganic phosphorus (IP) levels were found to be higher in cats receiving CRRT compared with the medical treatment group. In dogs, however, baseline values did not differ significantly between the treatment groups. Longitudinal analysis of animals receiving CRRT revealed a marked decrease in BUN, creatinine, and IP levels in cats over the course of treatment. A similar downward trend was observed in dogs; however, these changes were not statistically significant.

A comparison of pre- and post-treatment values confirmed that CRRT resulted in a marked reduction in BUN, creatinine and IP levels in cats; in the group receiving medical treatment, however, the changes were more limited. A measurable reduction in these parameters was also recorded following CRRT in dogs. When biochemical recovery times were examined using a Kaplan-Meier-style analysis, the mean recovery times for BUN and IP in cats were approximately 6 hours, whilst that for creatinine was approximately 5 hours. In dogs, creatinine levels improved more rapidly, with a median recovery time of approximately 1 hour; the times for BUN and IP values to return to normal were approximately 6 and 4 hours, respectively. The findings indicate that CRRT

leads to faster and more pronounced improvements in key renal biomarkers, particularly in cats. These results demonstrate that, in cases of severe renal failure, CRRT offers a potential therapeutic advantage over conventional medical treatment.

The findings of this study have shown that continuous renal replacement therapy (CRRT) is effective in reducing blood urea nitrogen (BUN), creatinine (CREA) and inorganic phosphorus (IP) levels, particularly in cats. In light of this, the following recommendations can be made:

1. In cats and dogs with severe azotaemia and advanced renal failure, particularly in cases where an adequate response to conventional parenteral fluid therapy cannot be achieved, it is recommended that CRRT be considered as a treatment option.
2. The study results have shown that CRRT in cats leads to a more rapid and marked improvement in biochemical parameters. It is therefore thought that the early application of CRRT in cats with chronic or acute kidney injury may improve clinical outcomes.
3. Although the trend towards biochemical improvement observed in dogs was not found to be statistically significant, clinically positive changes were observed. It is therefore recommended that studies with larger sample sizes be conducted to demonstrate the efficacy of CRRT in dogs more clearly.
4. In future research, evaluating not only biochemical parameters but also parameters such as clinical recovery, survival time and quality of life will contribute to a more comprehensive understanding of the clinical efficacy of CRRT.
5. Conducting experimental and clinical studies comparing different durations, flow rates and protocols of CRRT treatment may help to determine the most appropriate conditions for its administration.
6. Planning prospective studies with longer follow-up periods to assess the effects of CRRT on long-term renal function will make significant contributions to the literature.

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Research Article

Mechanical brushing, social interaction and gut microbiota analysis: Gut-brain axis directed gut microbiota in healthy calves before and after mechanical brushing

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Abstract

Mechanical brushes are well-recognized grooming tools widely adopted to improve the well-being of dairy cows and calves, and automated brushing has been reported to interact with social networks and behaviour in several species. Building on these observations, the present study hypothesized that calves that naturally investigate and use mechanical brushes may undergo shifts in gut microbiota composition, potentially mediated by social interaction and the associated skin–oral network. This field study was conducted under commercial farm conditions in apparently healthy calves aged 30–70 days that were socially bonded to their pen mates and used a mechanical brush at least daily. Infectious enteropathogens were excluded using point-of-care rapid diagnostic test kits. Faecal samples were collected rectally before brushing and after three weeks of mechanical brush use. Following extraction of faecal DNA, the V4 and V4–V7 regions of the 16S rRNA gene were amplified and sequenced on the Illumina iSeq100 platform (2 × 150 bp), and data were processed using QIIME 2 and Kraken2 with taxonomic assignment against the SILVA database. Before brushing, the microbial community was dominated by *Yersinia enterocolitica* (25.9%), others (26.7%) and *Pseudomonas tolaasii* (9.94%). After three weeks, the relative abundances of *Y. enterocolitica* and *P. tolaasii* fell to 10.1% and 5.75%, respectively, while taxa absent at baseline — *Haloquadratum walsbyi* (14.9%), *Methanobrevibacter smithii* (13.0%) and *Bacteroides uniformis* (5.50%) — became established. Increased proportions of *Phocaeicola vulgatus, [Ruminococcus] torque*s and *Pedobacter sp. HDW13* were also recorded, whereas *Burkholderia pyrrocinia, Defluviimonas sp. SAOS-178_SWC* and *Streptomyces sp. NBC_00536* were no longer detected. The reduction in potential pathogens, most notably *Y. enterocolitica,* is of particular interest given its role in enteric disease of cattle and its zoonotic importance. To the best of the author's knowledge, this is the first report associating mechanical brushing with gut microbiota alterations in calves, providing a rationale for enrichment-based interventions targeting gut health and welfare.

Keywords: Calf, environmental enrichment, gut microbiota, mechanical brushing, social interaction, 16S rRNA sequencing

Introduction

Given elevated scaling of dairy farms worldwide, dairy industrial processing exhibited a significant nutritional source for humans (Reynolds et al., 2013). Regarding past back ground of close era, human consumption of dairy products have gained elevated concern linked to welfare of dairy cows, and therefore calves, through its almost linked to quality of milk, raw milk delivery, dairy-related stuff and economic beneficiary (Barkema et al., 2015). High-welfare dairy farming facilities exhibited a pivotal participation within in the feasible progress for dairy production

(Crossley et al., 2021).

Relative researches participated out on weaned calves (Horvath & Miller-Cushon, 2019; Velasquez-Munoz et al., 2019; Zobel et al., 2017), presented that the elementary ambition modified the repetition and timing of mechanical brush intervention are elaborately interconnected with several issues [i.e. relevant temperature, encompassing noise concentration, and attainability for resources (Li et al., 2024).

Given mechanical brushing interacts with social network and behaviours among pigs (Högberg et al., 2025), horses (Lansade et al., 2022), the present investigator hypothesized that calves naturally investigating and exhibiting mechanical brushing behavior could cause switching in gut microbiota, probably related to social interacion. Besides another aim was to analyze whether alterations among gut microbiota could reflect skin-oral network.

Material and Method

Study demographics

This study was performed as prospective observational type, at field conditions. One of the involvement criteria, as was also reported above, was social network bounds previously described (Alıç Ural et al., 2022). Even if a calf was socialy bounded to the other relevant mates, and even prefer mechanical brush (Fig. 1), was participated. A total of 14 calves were composed of, of both sexes (9 female, 5 male) at the age of 30 to 70 days of age.

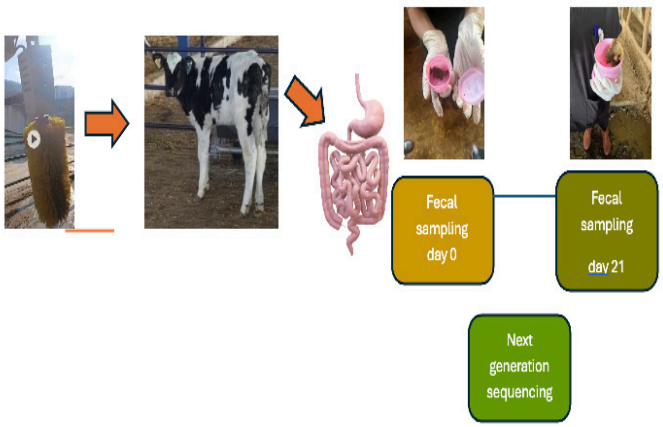


Figure 1. Schematic representation of present study, also evidencing hypothesis and methodology.

Ethical Approval

This study was ethically approved by the Animal Experiments Local Ethics Committee of Aydin Adnan Menderes University-HADYEK (Approval No: 64583101/2024/21).

Animals and Experimental Design

The animal material of the study consisted of 16 calves presented to with complaints of diarrhea. Infectious causes of diarrhea including *E. coli*, Rotavirus, Coronavirus, *Cryptosporidium spp.*, and *Clostridium perfringens* were excluded using point-of-care rapid diagnostic test kits (Anigen, Rapid BoviD-5 Ag Test Kit, Korea). Inclusion criteria were i) usage and preference of mechanical brushing, ii) social networking and usage of other objects available at farm, iii) at least daily interference with mechanical brush (not solely single usage) and iv) evidence of healthy conformation.

Fecal sampling

Fecal microbiota analytes were collected directly from rectal route by use of singe, sterile sampling containers, as shown at the bottom, along with several steps of methodological intervention.



Figure 2. Organization and team work at field conditions showing macro appearence of methodological steps for relevant fecal sampling.

Microbiota Analysis and 16S rRNA Sequencing

DNA was throughly extracted among fecal samples using a commercially available fecal DNA extraction

kit with regard to the manufacturer's instructions. The V4 and V4–V7 regions of the 16S rRNA gene were amplified by use of indexed primers. PCR amplification, purification, and library preparation were performed according to standard protocols.

Sequencing was conducted using the Illumina iSeq100 platform with paired end reads (2 × 150 bp). Raw sequencing data were processed using bioinformatics pipelines including QIIME 2 and Kraken2 for taxonomic classification.

Bioinformatics and Statistical Analysis

Sequencing data were analyzed for microbial diversity and taxonomic composition. Alpha diversity indices and beta diversity analyses were performed, and taxonomic classification was conducted using the SILVA database. Non-parametric data were expressed as median (min–max). Statistical significance was set at $p < 0.05$.

Results

Gut microbiota analytes

Before treatment, the most dominant species in the composition of the microbiota were *Yersinia enterocolitica* (25.9%), others (26.7%) and *Pseudomonas tolaasii* (9.94%). *Mycobacterium canettii, Klebsiella michiganensis* and *Streptomyces sp. NBC_00536* were presented in lower proportions.

The microbiota composition was significantly different after mechanical brushing. The proportions of *Yersinia enterocolitica* (10.1%) and *Pseudomonas tolaasii* (5.75%) decreased and species not detected before treatment, such as *Haloquadratum walsbyi* (14.9%), *Methanobrevibacter smithii* (13.0%) and *Bacteroides uniformis* (5.50%) appeared after treatment. Moreover, increased ratios of *Phocaeicola vulgatus*, [Ruminococcus] torques and *Pedobacter sp. HDW13* were observed. In contrast, *Burkholderia pyrrocinia, Defluviimonas sp. SAOS-178_SWC* and *Streptomyces sp. NBC_00536* were not detected in post-treatment samples.

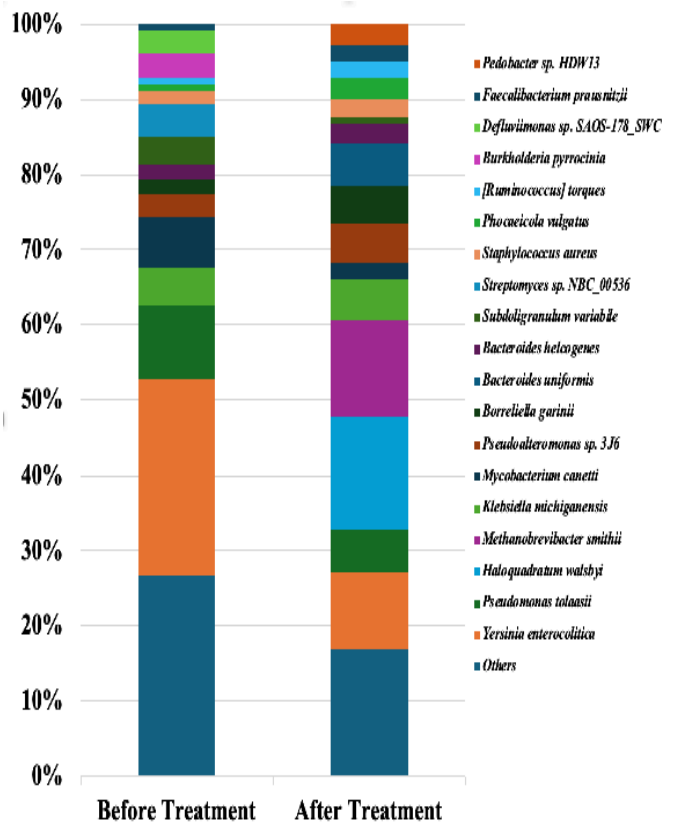


Figure 3. The basic structure of the bacterial community composition of two different sampling time, both at the species level, before and after mechanical brush treatment. Stacked bar graphs illustrate the relative abundances of bacterial species.

Discussion

In a previous research regarding dairy farming, social isolation of cattle was subjected. In that investigation it was aimed to detect even if environmental enrichment both by tactile (automated grooming brush) and visual (a mirror) stimulation might diminish stress. In a total of 18 cows exposed to 4 different isolation conditions of half an hour each; physiological and behavioural indicators of stress were analyzed. It was detected that the occurence of an automated grooming brush, a mirror or both within the isolation pen did not linked to decreased indicators of stress. In disaggreement with some other studies, diminished levels of stress among socially isolated heifers/cows (Mandel et al., 2019). In the present study altough the investigator did not analyzed stress biomarkers, the aim of her next and subsequent study would be thus aimed at this subject. Briefly, in the present study altered microbiome could be related to the animal

wellfare, and probably diminished stress by grooming via brushing. The present study was methodlogically involved social interaction, not isolation. Usage of mechanical brushing could alleviate stress and caused social interaction along with skin-oral network and communication. This could have helped microbiome shifting, as was shown in Fig. 3.

In a prior multidisciplinary study, social network analysis was performed in a group of 9 calves at the age of 10 to 16 weeks. Through full daily digital camera usage, interactions on each calf were detected and observed, field study lasted 4 weeks. At initial referral, only 2 was infected with giardiasis, whereas 3 other calves were also infected on the second week. All other elevant calves were additionaly infected at the end of trial, associated with close contact. In summary it was concluded that social networking played significant predisposing factor for giardiasis spread to those of calves enrolled (Alıç Ural et al., 2022). In the present study this social network modality was similarly adopted through usage of mechanical brushing to those of apperantly healthy calves. On the opposite direction and results, in comparison to that study (Alıç Ural et al., 2022), mechanical brush usage caused diminished relative abundaces of some selected and detected pathogens, detected at this study (Fig. 3).

In the present study prior to mechanical brush usage at day 0, the vast majority of bacterial species involved *Yersinia enterocolitica* (25.9%), others (26.7%) and *Pseudomonas tolaasii* (9.94%). Yersiniosis is one of the vast majority marked intestinal disease agent in association with *Y. enterocolitica*, influencing humans and and also cattle and relevant species (Chlebicz & Śliżewska, 2018; Saleh & Zenad, 2018). Several different social and environmental issues were linked to *Y. enterocolitica* spread, resulting with marked public health/economic problems (Curtis et al., 2014; Råsbäck et al., 2018). In a previous research 1020 samples [660 rectal swabs collected from live cattle] exhibited *Y. enterocolitica* isolation 3 out of 15 examined cattle herds. Isolation rates were as follows 14.7%, 7.4% and 5.5% of heifers, calves and adult cows, respectively (Łada et al., 2023). On the other hand altough Pseudomonadales was evident in healthy calves vs. diarrheic ones (Zhang et al., 2025), *Pseudomonas tolaasii* relative abundance was 9.94% prior to mechanical brush usage, where declined to 5.75%. Moreover mechanical brush usage caused diminished *Y. enterocolitica* abundance from 25.9% to 10.1% (Fig 3).

The microbiota composition was significantly altered following 3 weeks of mechanical brushing. Some species such as *Haloquadratum walsbyi* (14.9%), *Methanobrevibacter smithii* (13.0%) and *Bacteroides uniformis* (5.50%), not previously detected previously, existed after mechanical brushing. Furthermore *Phocaeicola vulgatus*, *[Ruminococcus] torques and Pedobacter sp. HDW13* were existed, whereas *Burkholderia pyrrocinia, Defluviimonas sp. SAOS-178_SWC* and *Streptomyces sp. NBC_00536* were disappeared through 3 weeks of mechanical brushing (Fig 3).

Conclusion

In conclusion the present prospective observational study concludes that microbial alterations could have critical role within the wellfare of calves. This finding, altough was not supported by biomerkers of relevant, was based on personnal observations recorded (live weight gain, disease free status of at least stuıdy period etc.). Obtained findings provide insights for designing purposed interventions for modifying gut health among calves. Mechanical brushing for at least 3 weeks of usage, caused altered microbiome among calves enrolled herein.

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